(54) Title: MENINGOCOCCAL VACCINE

(57) Abstract

The present invention relates to an anti-meningococcal vaccine, particularly for group-B serotype meningococcus. The invention provides antigenic peptide ligands which can act as an immunogen capable of eliciting an immune response to produce antibodies against the capsular polysaccharide of group-B meningococci (CPS-B).

Meningococcal Vaccine

The present invention relates to an anti-meningococcal
vaccine, particularly for group-B serotype meningococcus.
The invention provides antigenic peptide ligands which can
5   act as an immunogen capable of eliciting an immune
response to produce antibodies against the capsular
polysaccharide of group-B meningococci (CPS-B).   The
immunogen may be in the form of a polypeptide or in the
form of a conjugate of such a polypeptide coupled to a
10   carrier molecule such as a protein or in the form of a
peptide displayed upon a virus particle (ie. a virion),
for example a bacteriophage. The immunogen may be used in
treatment of or prophylaxis against group-B meningococcal
infection. The invention also provides
15   anti-group-B-meningococcal antibodies for use in treatment
and/or prophylaxis. In addition, the invention relates to
methods for producing such immunogens, antibodies and
vaccines and relates to their use in methods of treatment
and prophylaxis.

20   Infection by group-B Meningococci (Neisseria meningitidis)
is the major cause of bacterial meningitis in the UK, EC
and worldwide[1-9].   In the UK approximately 60% of the
bacterial meningitis cases are due to group-B
meningococci.   The majority of cases occur in infants of 3
25   months to 3 years of age. Despite the best efforts of
medical staff, and the rapid use of antibiotic and other
intervention strategies the mortality rates in the UK
still hover around the 2-6% for meningococcal meningitis
and 14-50% for meningococcal septicaemia.   There is also a
30   considerable amount of post-infection morbidity in

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survivors.   There is therefore a requirement for a vaccine
to group-B meningococci.   Indeed vaccines against other
serotypes of meningococci, including groups A, C and Y
have been successfully produced[10-12]. These vaccines all
5   utilise capsular polysaccharide material to elicit a
protective immune response, but the responses are
serogroup specific and to date there has been little
success in efforts to produce an effective vaccine to the
B-group meningococci, using similar approaches[13].   The
10   current status of attempts to obtain a B-group
meningococcal vaccine has been reviewed by Romero et
al[14(a)], and the background to vaccines against
meningococcal diseases has been reviewed by Ala'aldeen et
al[14(b)].

15   Unlike the capsular polysaccharide of other serogroups,
that of the B-group meningococci is very poorly
immunogenic. This is probably due to a number of factors,
including the sensitivity of the polysaccharide to
degradation by host enzymes and also the similarity of the
20   polysaccharide structure to carbohydrate components
present within the host tissues[15-17]. The capsular
polysaccharide of group-B meningococci is a homopolymer of
repeated residues of α-(2-8)-linked oligomers of sialic
acid: more specifically it is a linear polymer of $\alpha(2'-8)$
25   linked '-N-acetylneuraminic acid residues[18-20] (2'-8 'NANA).
This particular form of polysaccharide capsule material is
found only in group-B meningococci, E. coli K1 strain,
which is also a human pathogen, Pastuerella haemolytica, a
sheep pathogen and Moraxella nonliquefaciens a
30   non-pathogenic commensal[21]. When isolated from E. coli K1
the polysaccharide is referred to as "Colominic acid".

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Efforts have to a large extent turned away from the use of
capsular polysaccharide alone as a potential vaccine
material, and concentrated on other components of
bacterium (such as fimbriae, outer membrane proteins
5   (OMPs) etc.), without noticeable success in terms of
potential vaccines.

The general consensus of data concerning A, C, Y and W135
serogroup specific meningococcal vaccines already
available suggests that capsular polysaccharide material,
10   used as a vaccine, elicits protective immune responses.
Indeed immunoglobulins which bind the group-B capsular
polysaccharide (both IgM and IgG classes) can be shown to
have bactericidal activity in vitro and protect against in
vivo challenge with lethal doses of *N. meningitidis* or *E.*
15   *coli* K1. However, the capsular polysaccharide of group-B
meningococci appears unsuitable for effective use as a
vaccine material because it is non-immunogenic or very
weakly immunogenic even when coupled to carrier proteins.

An extract from the conclusions of the Romero survey [14(a)]
20   reads as follows:

        "The search for an ideal antigen as a potential
        candidate for an *N. meningitidis* B vaccine has made
        the outer surface of this organism one of the most
        widely studied cell coats. Not only have the results
25   advanced our understanding of the different
        mechanisms of associated virulence and pathogenicity,
        but the research techniques developed have often been
        applicable to the development of vaccines that
        protect against other capsulated bacterial pathogens.

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Hence, one of the *H. influenzae* type B vaccines
consists of a capsular polysaccharide conjugate to
meningococcal outer membrane proteins that are used
for their special immunogenic properties.

5   For an antigen to be considered a vaccine candidate
for the entire serogroup B, two fundamental
requirements must be fulfilled: it must be highly
conserved among the different strains, and it must
induce bactericidal antibodies. The latter is of
10   particular importance in protection against *N.*
meningitidis. To date, it does not seem that these
characteristics can be found in a single candidate
vaccine, so a multifaceted approached to the problem
is needed in order to meet this goal".

15 An extract from the conclusions of the Ala'aldeen survey
[14(b)] reads as follows:

"It is evident that there has been considerable
activity in the meningococcal vaccine field over the
past few years and there is now room for some
20   optimism. The advances in the development of
polysaccharide conjugate vaccines should eventually
lead to much more effective vaccines against Group A
and C disease, in particular to vaccines that will be
effective in the youngest children who are indeed
25   most at risk. However, the problems of a group-B
meningitis vaccine remain."

The current state of the art does not allow the ab-initio
design of antigenic ligands, even given an antibody

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structure (such as an X-ray crystallographic structure).
In the specific case of peptides mimicking the relevant
conformational structure of the group-B meningococcal
polysaccharide, ab-initio design would be particularly
difficult since there is no knowledge available as to what
amino acid residues or sequences might be suitable as
mimetics of this structure. Although studies by other
investigators (Hoess, Scott, Devlin) have identified
peptides mimicking monosaccharide or oligosaccharides,
these data are not sufficient to make any useful
predictions about the residues, sequences or structures
of peptides that might bind an anti-group-B polysaccharide
antibody.

Conventional approaches to the development of vaccines
against infectious disease use the infectious agent in an
inactivated or attenuated form, or some part of that
agent, as a basis for the vaccine. The approach we have
adopted uses instead an antibody which embodies a surface
which is complementary to some site on the agent (in terms
of shape, charge etc.). According to this method, the
antibody is used (metaphorically) as a mould to fashion
peptide ligands which represent the corresponding site on
the infectious agent. Preferably the site, and the
corresponding antibody comprise a neutralising site and a
neutralising antibody respectively - such that attachment
of the antibody to the infectious agent inactivates the
infectious agent or toxic products of that agent.

The process of generating peptides representative of the
infectious agent is accomplished using libraries of
synthetic or phage-displayed random peptides. Members of

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peptide libraries that fit the antibody well comprise
candidates for incorporation into vaccines, for example,
in the form of peptide/carrier protein conjugates, or in
the form of phage-peptide constructs.

5   In a first aspect the invention provides a pharmaceutical
compound which includes a chemical composition capable of
adopting a structure essentially equivalent to the
pharmacophoric pattern of a section of the capsular
polysaccharide of Neisseria meningitidis group B,
10   optionally together with a pharmaceutically acceptable
carrier or excipient for use as an anti-group B capsular
polysaccharide immunogen.

In a second aspect the invention provides peptides which
include an amino acid sequence capable of adopting a
15   structure having an pharmacophoric pattern essentially
equivalent to the pharmacophoric pattern of a section of
the capsular polysaccharide of Neisseria meningitidis
group B.

In a third aspect the invention provides antigenic peptide
20   ligands which cross react with antibodies against the
capsular polysaccharide of group B meningococci (CPS-B)
which peptides include 1 or more copies of a motif which
consists of a ring aromatic moiety, a spacer moiety, and a
moiety having negative charge. In addition to these 3 key
25   moieties there is a further optional moiety which is a
possible additional hydrogen bond donor/acceptor. Such
peptides may have two or more of said motifs which may
repeat sequentially or may repeat in an overlapping frame.

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In a fourth aspect the invention provides an antigenic peptide which includes an amino acid sequence and structure essentially equivalent to the pharmacophore defined as follows:

the pharmacophore includes at least 3 chemical features; a Ring Aromatic, a Negative Charge and a Hydrogen Bond Acceptor or Donor feature. In addition to these 3 key chemical features there is a further optional feature which is a possible additional hydrogen bond donor/acceptor. The key features are further defined as follows:

(1A) the hydrogen bond acceptor feature matches the following atom types or groups of atoms which are surface accessible;

- sp or sp^2 nitrogens that have a lone pair and a charge less than or equal to zero
- sp^3 oxygens or sulphurs that have a lone pair and charge less than or equal to zero
- non-basic amines that have a lone pair;

(1B) the hydrogen bond donor feature has the same chemical characteristics as the hydrogen bond acceptor except that it also includes basic nitrogen (there is no exclusion of electron-deficient pyridines and imidazoles);

this feature matches the following atom types or groups of atoms;

- non-acidic hydroxyls
- thiols
- acetylenic hydrogens
- NH moieties (except tetrazoles and trifluoromethyl sulfonamide hydrogens);

(2) the negative charge feature is defined as a negative charge not adjacent to a positive charge; and

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(3) the ring aromatic feature is defined as an
aromatic moiety which may be replaced by amino acid
residues having hydrophobic character such as the amino
acid residues selected from the following group;

5   methionine, alanine, leucine, isoleucine, valine,
proline; and

(ia) the hydrogen bond acceptor is represented by a
vector function consisting of two spheres;

the smaller sphere (at least 1.6Angstroms radius up

10   to 2.6 Angstroms) defines the centroid of the hydrogen
bond acceptor on the ligand while the large sphere (at
least 2.2Angstroms radius up to 2.6 Angstroms) defines the
projected point of the hydrogen bond acceptor from the
receptor;

15   these two spheres are at least 3.0Angstroms apart;

(ib) the hydrogen bond donor is represented by a two
sphere vector function as (ia) above;

(ii) the negative charge is represented by a sphere
at least 1.6Angstroms radius (up to 2.6 Angstroms); and

20   (iii) the ring aromatic is represented as two equal
size spheres (at least 1.6Angstroms radius up to 2.0
Angstroms) whose centroids are 3.1Angstroms apart;

one sphere corresponds to the position of an aromatic
ring moiety and the other to the projected point of the

25   electron pi stacking of the aromatic ring system;

and wherein the tolerances on all distances between
these features is +/- 0.5 Angstroms and the geometric
angles +/- 20 Degrees and said distances and angles are
shown in figure 1 or figure 2.

30   In a fifth aspect the invention provides antigenic
peptides of 3 to 25 amino acids of the general formula:

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X-B-C-D-Y

wherein

X is any group of amino acids of length P;

D is any hydrophobic amino acid group;

5   C is any amino acid;

B is any negative residue;

Y is any group of amino acids of length Q; and

P and Q may be zero and P + Q is less than or equal to 22.

Note that since X and Y may be absent or identical the

10   *above defined "motif" may be read in either direction in*

many circumstances.

These peptides preferably have 6 to 20 amino acids, most

preferably 9 to 15 amino acids.

Preferably P + Q is less than or equal to 17, most

15   preferably P + Q is less than or equal to 8.

Preferably X contains His.

Preferably D is Tyr.

Preferably C is an uncharged amino acid; more preferably C

is Ser or Thr, most preferably C is Thr.

20   Preferably B is an acidic residue, more preferably B is

Asp or Glu, most preferably B is Glu.

Preferably Y contains His.

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Thus a preferred embodiment of the invention has the general formula

   X(containing a His)-Tyr-Thr-Glu-Y(containing a His).

In a sixth aspect the invention provides antigenic peptides of 5 to 25 amino acids of general formula:

                         X-ABCDE-Y

wherein

A is any amino acid;

E is any amino acid;

X is any group of amino acids of length P;

Y is any group of amino acids of length Q; and

P & Q may be zero and P + Q is less than or equal to 20 and B,C,D are as defined as above.

Note that since X and Y may be absent or identical the above defined "motif" may be read in either direction in many circumstances.

Preferably the peptide is 6 to 20 amino acids, most preferably the peptide is 9 to 15 amino acids.

Preferably B is Asp or Glu, most preferably B is Asp.

Preferably A is Met.

Preferably E is His.

Preferably P + Q is less than or equal to 15, most preferably P + Q is less than or equal to 10.

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Thus a preferred embodiment of the invention has the
general formula X-Met-Asp-Arg-Tyr-His-Y [SEQ ID NO:33].

Compounds and peptides of the invention (either alone or
as a conjugate) may advantageously be immunogenic.

5   For example according to the invention there is provided
an immunogenic ligand which comprises a synthetic
polypeptide selected from compounds of sequence SEQ ID
NO:1:
                    TIPLWFDDEIEVMIY
10   and analogue and homologue derivatives thereof by virtue
of one or more amino acid addition, deletion,
substitution; together with terminal functional
derivatives thereof; or an immunogenic ligand which
comprises a synthetic polypeptide selected from compounds
15   of sequence SEQ ID NO:2:
                    GDNFESYACVDTPCS
and analogue and homologue derivatives thereof by virtue
of one or more amino acid addition, deletion,
substitution; together with terminal functional
20   derivatives thereof.

For example the immunogenic ligand may be the
pentadecapeptide of SEQ ID NO:1
                      TIPLWFDDEIEVMIY
or the pentadecapeptide of SEQ ID NO: 2
25   GDNFESYACVDTPCS
and terminal functional derivatives thereof.

In certain embodiments of the invention the immunogenic
ligand is conjugated to a carrier.

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In certain embodiments of the invention the immunogenic
ligand comprises a bacteriophage which encodes and is
capable of expressing a synthetic polypeptide as defined
above.

5   In a seventh aspect the invention provides an antibody
produced by an immune response to an immunogenic ligand as
defined above which antibody specifically recognises and
binds the synthetic polypeptide of the ligand and also the
group-B meningococcal Capsular Polysaccharide (CPS-B).

10   The invention also provides a pharmaceutical composition
containing as active ingredient at least one ligand or one
antibody as defined above, and optionally including an
adjuvant or excipient, for use in the treatment of
infection by group-B meningococcal bacteria or for use in
15   prophylactic prevention of infection by group-B
meningococcal bacteria.

This invention also provides a method of treatment or
prophylaxis of infection with group-B meningococcal
bacteria, which comprises administering an effective
20   amount of a composition, antigen, ligand or antibody as
defined above.

Amino acids and amino acid residues herein represent D and
L amino acids, their analogues or derivatives.

The invention will now be described by way of example only
25   with reference to the accompanying figures in which:

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Figure 1 is a geometric representation of pharmacophore 1 in which position 1 indicates the ring aromatic, position 2 indicates the location of the negative charge, and position 3 indicates the location of either the hydrogen bond donor or the hydrogen bond acceptor;

Figure 2 is a geometric representation of pharmacophore 2 in which position 1 indicates the ring aromatic, position 2 indicates the location of the negative charge, and position 3 indicates the location of either the hydrogen bond donor or the hydrogen bond acceptor;

Figure 3 corresponds to the figure 1 pharmacophore wherein positions 1, 2 and 3 are represented by spheres based on the centroids of the chemical features;

Figure 4 corresponds to the figure 2 pharmacophore wherein positions 1, 2 and 3 are represented by spheres based on the centroids of the chemical features;

Figure 5 shows the pharmacophore of figure 3 superimposed with two palindromic molecular structures represented by the sequences YTE and DRY.

Figure 6 shows the pharmacophore of figure 4 superimposed with two palindromic molecular structures represented by the sequences DRY and YTE.

Figure 7 shows the pharmacophore of figure 3 superimposed with a molecular structure represented by the sequence MDRYH;

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Figure 8 shows the pharmacophore of figure 4 superimposed
with a molecular structure represented by the sequence
MDRYH;

Figure 9 shows the pharmacophore of figure 3 superimposed
5   with a molecular structure of the dye remazol brilliant
violet;

Figure 10 shows the pharmacophore of figure 4 superimposed
with a molecular structure of the dye remazol brilliant
violet;

10   Figure 11 shows the pharmacophore of figure 3 superimposed
with a molecular structure of the capsular polysaccharide
of group-B meningococci;

Figure 12 shows the pharmacophore of figure 4 superimposed
with a molecular structure of the capsular polysaccharide
15   of group-B meningococci;

Figure 13 corresponds to figure 1 but shows the presence
of an additional hydrogen bond donor/acceptor;

Figure 14 corresponds to figure 2 but shows the presence
of an additional hydrogen bond donor/acceptor;

20   Figure 15 corresponds to figure 3 but shows the presence
of an additional hydrogen bond donor/acceptor;

Figure 16 corresponds to figure 4 but shows the presence
of an additional hydrogen bond donor/acceptor;

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Figures 17 through 21 correspond to figures 5, 7, 9 and 11
but show the presence of an additional hydrogen bond
donor/acceptor;

5   Figure 22 shows results of probing a primary synthetic
peptide library with a human anti-group-B polysaccharide
antibody (IgM^{Nov});

Figure 23 shows a combinational pharmacophore based on
pharmacophores 1 and 2 superimposed on eachother. The
resulting more detailed pharmacophore has features of both
10   pharmacophores 1 and 2.

Figure 24 shows the pharmacophore of figure 23 and the
centroids are labelled.

Figure 25 shows the absolute positions of the centroids of
the pharmacophore shown in figure 23.

15   Figures 26 to 29 show the distance and angle constraints
of the centroids shown in figures 24 and 25.

Figure 30 demonstrates that the antigenic core of the
GDN... peptide (ESYACVDTPCS) maps to a combined version of
the pharmacophore. Thus, critical side chains map to all
20   of the features of this pharmacophore: E and D to the
negative ionizable features; Y to the aromatic/hydrophobic
feature and the hydroxyl of the Y side-chain maps (in this
instance) as a hydrogen bond donor. The antigenic
importance of other residues in the peptide that do not
25   map to identified features of the pharmacophore is likely
to be attributable to their effect on the conformation of

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this peptide, thereby affecting the disposition of these
critical side-chains.

Figure 31 depicts the mapping of the combined
pharmacophore to the antigenic core region of peptide
5   TIP..., namely DEIEVMIY. In this mapping D1 and E4 satisfy
the negative ionizable features, Y satisfies the ring
aromatic/hydrophobic feature, a backbone N-H providing the
hydrogen-bonding element (donor in this instance).

Figure 32 depicts the mapping of methylmalonyl coenzyme-A
10   to the combined pharmacophore. Note that all of the
features are satisfied.

Figure 33 shows the less active compound, D-luciferin, of
IC50 36μM fitting the combined pharmacophore. Note that in
this mappinng, not all features are mapped, consistent
15   with its lower activity.

Figure 34 depicts an alternative mapping of D-luciferin to
the combined pharmacophore. As before, not all features
are mapped. The ability of D-luciferin to map in two
distinct modes to the pharmacophre may explain the fairly
20   good antigenic activity of this relatively small compound.

Figure 35 shows that the polysaccharide is capable of
satisfying several (4/5) structural features of the
combined pharmacophore. Other mappings were found to be
possible (not shown#). Surprisingly, in this particular
25   mapping the negative ionizables fit well with alternate
carboxylates of the polysaccharide (and not with adjacent
carboxylates as might be anticipated) while both of the

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hydrogen bonding features (having a shared point of origin) are also satisfied. Other mappings of the pharmacophore (not shown) were found to be possible, including ones in which the aromatic/hydrophobic feature was occupied by the six-membered ring of the saccharide monomer. Although this ring is not strongly hydrophobic in character, it may at least be compatible with occupying a hydrophobic site on the antibody. The existence of a plurality of mappings of the combined pharmacophore to this structure may reflect an ability of the antibody to engage multiple aspects of the helical structure of the polysaccharide, meaning that antigens identified by the pharmacophore may be particularly effective immunogens.

Figures 36 and 37 show tables illustruating the degree of reaction of antibody with peptides synthesized on membrane assessed using the method of Gao and Esnouf (1996). Scoring of reaction is as follows: '0' = no change (equivalent to signal generated with original peptide sequence); -1modest decrease; -2 strong decrease; -3 very strong decrease; # signal abolished; $ same as original sequence. Positive numbers represent an increase in signal. Deviations of 2 units or greater from the original signal are considered to be significant (i.e. 2 or -2). Blank cells indicate 'not determined'.

Figure 38 shows inhibition of binding of human anti-polysaccahride antibodies IgM Nov and 64V to solid phase poysaccharide by solution phase haptenic antigens of the diadenosine polyphosphate homologous series.

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Figure 39 shows graphs of the binding of IgM Nov to Ala
scanned mutants of GDN...

Figure 40 shows the list of compounds fitting the
combinatorial pharmacophore and the training set for the
5   Combinatorial pharmacophore.

(For all of Figures 3 through 12, 15 through 21 and 23
through 35 the Figures are attached in two versions: a) in
black and white and b) in colour print).

To generate an appropriate antigenic peptide from a
10   phage-peptide library an antibody is used to select phage
expressing the peptide from mixtures of phage clones
expressing a great variety (up to 100 million) of unique
peptide sequences, essentially as described by Scott and
Smith (Science, 1990). The identity of the peptide ligands
15   is then deduced by sequencing of the recombinant DNA
encoding the insert.

In the case of synthetic peptide libraries, suitable
peptides can be generated most conveniently by screening
of complex mixtures of peptides synthesised as pools, for
20   example in the form of a solid phase array (Gao et al 1996
in press) as described below.

Panning of hexapeptide libraries on pIII (coat protein
encoded by phage gene-III) of filamentous phage according
to the method of Scott and Smith (1990) was performed
25   using IgM^{Nov}, a well-characterised human antibody against
the capsular polysaccharide of the group-B meningococci
which is both bactericidal and protective in the infant

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rat model. The resulting clonal population of
peptide-expressing phage selected by the antibody did not
however exhibit any thematic or consensus motif among the
peptides sequenced (see e.g. SEQ ID NOS:12-24). Also, the
selected clonal population of phage did not exhibit any
evidence of specific binding to the selecting antibody on
solid phase in ELISA. Further studies of the binding
properties of individual phage clones demonstrated only
weak specific binding of certain examples of the antibody
selected clones, with signal to background ratios in the
region of 2:1 or less. Such peptides, having such low
avidity for the selecting antibody were not deemed
suitable candidates for use in vaccine development.
Moreover, the absence of thematic sequences for these
peptides made it difficult to determine what
physico-chemical attributes of the peptides were
responsible for antibody binding, or mimicry of the
polysaccharide. Moreover, the reactivity of synthetic
forms of these peptide ligands in solution phase with
IgM^{Nov} was weak or undetectable by competition ELISA with
polysaccharide on the solid phase.

Given the failure of the phage gene-III system to generate
good ligands for IgM^{Nov}, and the known requirement of
anti-group-B polysaccharide antibodies to recognise a
relatively large conformational feature of the
polysaccharide eg. comprising several of the sialic-acid
monomers (Kabat), it was deemed desirable to use larger
peptides. Since the numerical equivalence of amino-acid
residues with sialic acid monomers was uncertain, we chose
a library of 15mers to give a margin of error. This second
phage system comprised a display library system whereby

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random peptides were displayed fused to the major coat
protein - pVIII, derived from gene-VIII.

Selection of the hybrid phage random library of 15mers
with IgM^{Nov} gave rise to a polyclonal population of

5   selected phage which did exhibit specific binding to the
selecting antibody on ELISA (in contrast to experience
with the 6mer library in gene-III).

Binding studies on the isolated phage clones revealed two
which gave strong signal to background ratios, of the

10   order of 10:1 on ELISA. These phage clones were quite
different in primary sequence, one containing a putative
disulphide bond (GDN...:SEQ ID NO:2), the other
(TIP...:SEQ ID NO:1) exhibiting a strong helical
propensity according to secondary structure predictions.

15   Despite the superior binding characteristics of these
phage clones isolated from the 15mer libraries in
gene-VIII, the dearth of sequences isolated in these
experiments and their lack of a common motif still failed
to identify what physicochemical attributes (e.g. what

20   residues, sequences etc.) were responsible for peptide
binding to the antibody, or for antigenic mimicry of the
polysaccharide. Moreover, modelling of the peptides using
energy minimisation techniques also failed to identify
candidate structural features which might explain their

25   binding to an anti-polysaccharide antibody.

Given the strong specific binding of the selected phage
clones expressing 15mer ligands for IgM^{Nov}, even under
conditions of monovalent display, it was surprising to
find that the synthetic versions of these peptide

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sequences exhibited only weak activity in competitive
ELISA where the solution phase peptides were tested for
their ability to inhibit antibody-binding to solid phase
polysaccharide. Thus, although the phage-displayed 15mers

5   were good ligands, their antibody binding was dramatically
weakened once dissociated from the context in which they
were selected (i.e. that of the phage coat). These
findings of the context-sensitive antigenicity of phage
gene-VIII displayed polysaccharide-mimetic peptides

10   suggest the imposition of significant structural
constraint by the phage gene-VIII coat environment, and
concur with much of our experience with antibodies against
proteinaceous antigens of infectious agents. These
findings further suggest that that the structure of

15   peptides mimicking the group-B polysaccharide, rather than
merely the residues they contain or the sequence in which
these residues occur, is fundamentally important to their
polysaccharide mimicry. However, these findings give
little clue as to what that structure is. Studies were

20   therefore initiated with synthetic peptide libraries in
order to gain insight into the amino-acid residues,
sequences and peptide structures that determine peptidic
mimicry of the group-B polysaccharide.

Array libraries of synthetic peptides were constructed.
25   Such libraries are constructed as an array of spots on a
membrane - each spot conforming to a prescribed generic
formula, e.g. XOXOXXXX [SEQ ID NO:3] - where 'X'
represents a mixture of all 19 proteinogenic amino acid
residues (excluding cysteine) and where 'O' represents a

30   fixed position in the sequence wherein the identity of the
amino acid residue at that position is known. By arranging

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the spots in a 19 x 19 grid it is possible to explore all
possible permutations (361) of the fixed residues, i.e.
XAXAXXXX [SEQ ID NO:4], XAXDXXXX [SEQ ID NO:5] etc., where
each spot contains over 1 million unique explicit peptides
and the library contains about 2 x 10^{13} sequences in all.

The probing of such an array library with Nov serum
(containing IgM^{Nov}) followed by an anti-mu-chain specific
peroxidase conjugate revealed selective staining of
certain spots upon exposure of the blot to X-ray film in
the presence of a luminescent peroxidase substrate. The
strongest of these signals represented the intersections
of column Y with row E (representing XYXEXXXX [SEQ ID
NO:6]) and of column D with row Y (representing XDXYXXXX
[SEQ ID NO:7]). Interestingly, these signals provide a
degree of mutual confirmation since they exhibit an
imperfect form of palindromy -YXE- being analogous to
-DXY- (D and E each have negatively charged side chains).
Moreover, the truly palindromic representations of the
strongly recognised sequences -YXE- and -DXY- (i.e. spots
containing -EXY- and -YXD-) also gave rise to positive
signals, albeit weaker ones. Refer to figure 24. These
findings clearly implicate the residues Y and D/E as
critical for the binding the anti-group-B polysaccharide
antibody IgM^{Nov} to peptides. Further studies with secondary
synthetic libraries (see below) also demonstrate that
these signals are indeed attributable to IgM^{Nov}, since they
are abolished by preabsorption of the serum with Colominic
acid (i.e. α-(2,8)-linked polysialic acid, the common
capsule material of E.coli K1 and the group-B
meningococci). Moreover, these findings also demonstrate
stereospecificity of binding, since YXE is not

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stereochemically equivalent to EXY and DXY is not
stereochemically equivalent to YXD.

In the above studies with a primary synthetic peptide
library we chose arbitrarily to fix the spacing and
distribution of fixed residues in the library template in
the format XOXOXXXX [SEQ ID NO:3], since our previous
studies with anti-protein antibodies (BG) had proven this
spacing to be suitable. Since there was no priori reason
to suppose that such a format would be optimal for the
anti-polysaccharide antibody, we also chose to explore
alternative spacing and positioning of the fixed residues
in the generic template to determine the optimal spacing
and positioning for each permutation of fixed residues.

Further libraries were therefore constructed to explore
alternative spacing and positioning for all 361
permutations of fixed residues. Templates of the following
generic formulae, OOXXXXXX [SEQ ID NO:8], OXOXXXX [SEQ ID
NO:9], XOXXOXXX [SEQ ID NO:10] and XXXOXXOX [SEQ ID
NO:11], were constructed exploring spacing of 0, 1 and 2
with various positioning, in a search for further binding
motifs. This revealed the following additional binding
motifs for IgM^{Nov}:

1.	REXXXXXX	[SEQ ID NO:12]
2.	TDXXXXXX	[SEQ ID NO:13]
3.	TEXXXXXX	[SEQ ID NO:14]
4.	HXDXXXXX	[SEQ ID NO:15]
5.	DXHXXXXX	[SEQ ID NO:16]
6.	LXHXXXXX	[SEQ ID NO:17]
7.	XDXHXXXX	[SEQ ID NO:18]

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8.   XXDHXXXX [SEQ ID NO:19]

9.   HXXEXXXX [SEQ ID NO:20]

10. XHXXEXXX [SEQ ID NO:21]

11. XDXXHXXX [SEQ ID NO:22]

12. XXHXXEXX [SEQ ID NO:23]

13. XXXHXXEX [SEQ ID NO:24]

This study confirmed observations made earlier in this
document that histidine (H) was strongly implicated as a
determinant of binding, with certain strict provisos.
Also, the simultaneous presence of an negative (e.g.
acidic) residue is mandatory (D or E), with a preferred
spacing of two residues between the negative residue and
H, although a spacing of one residue is tolerated. Whilst
tyrosine (Y) plus a negative (e.g. acidic) residue were
found to be important, palindromy was either not
tolerated, or was tolerated only poorly. Thus, whereas
HXXE [SEQ ID NO:25] was positive, EXXH [SEQ ID NO:26] was
not. Likewise although DXXH [SEQ ID NO:27] was positive,
HXXD [SEQ ID NO:28] was not. Furthermore, when separated
by a single residue, this study reveals poorly tolerated
palindromy of HXD which is preferred over DXH.
Interestingly however, neither HXE nor EXH was tolerated.

These findings demonstrate stringent requirements for the
spacing of H with a negative (e.g. an acidic) residue as
determinants of binding. They also further underscore the
stereochemical specificity of the interaction of the anti-
polysaccharide antibody with peptide antigens. Further
studies described below using secondary synthetic
libraries, confirm and extend the findings of the primary
and secondary library studies in implicating histidine and

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tyrosine as determinants of binding in combination with
acidic and other amino acids at specified positions. These
latter studies further demonstrate that the stringency of
spacing of histidine and the acidic side chain of glutamic
or aspartic acid becomes less important when other
residues are allowed to make a major contribution to
binding.

Secondary synthetic libraries were constructed according
to the spot-method (Gao et al in press) based upon the
major leads found in the primary library studies, to give
templates of the following generic formulae: XYXEXXXX [SEQ
ID NO:29] and XDXYXXXX [SEQ ID NO:30]. A marked reactivity
of IgM^{Nov} was observed with histidine-containing peptides
in each of these libraries. Comparison of these results
with those of the primary library studies (above)
demonstrate that this histidine-dependent reactivity is
contingent upon the presence and appropriate distribution
of both tyrosine and an acidic residue, since most of the
37 histidine-containing pools represented by spots in each
of the five primary libraries (which explored spacing etc:
see above) were unreactive with the antibody. With this
proviso, in general terms, the requirement for histidine
was largely position-independent in these secondary 8-mer
libraries. The single exception to this rule was a
preference for histidine at position 4 in the secondary
library of template XDXYXXXX [SEQ ID NO:30](i.e. XDXYHXXX
[SEQ ID NO:31] (which contains a motif -DXYH- SEQ ID NO:
32). Figure 25 shows the results of probing two secondary
synthetic peptide libraries of generic formulae XYXEXXXX
(upper panel) and XDXYXXXX (lower panel) with a human
anti-group-B polysaccharide antibody. Not all signals

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were inhibited by preabsorption of the antibody with
polysaccharide (Colominic acid), the ones which were
strongly inhibited are indicated. The preferred linear
peptides can be inferred from this data as -YT/sE-[H]
(upper) where T/s represents a preference for threonine
and a tolerance of serine. The brackets around "H"
represent unspecified position in the sequence. The
dashes represent occurrence internally in the sequence.
The other preferred peptide was MDXYH, where the presence
of histidine at position 5 was preferred. This finding
confirms the observations made with the primary libraries
which explored positioning and spacing of the fixed 'O'
residues. These studies implicated -DXXH- [SEQ ID NO:27]
as a positive motif, consistent with the XDXYHXXX [SEQ ID
NO:31] peptide found in the secondary library study.

The findings of the secondary synthetic library studies
which used linear peptides can be summarised as two
motifs, namely: -YT/sE-[H] and MDXYH, where dashes
indicate but do not exclude occurrence non-terminally in a
peptide sequence, and where parenthesis indicates non-
specified position in the sequence.

The identified motifs are unexpectedly short - given the
requirement of IgM[Nov] to recognise higher oligomers of
sialic acid (9-unit oligomers being optimal). This may be
because these peptides bind in an extended conformation,
whereas the polysialic acid binds in a helical
conformation (Brisson et al). Alternatively, they may
reflect the binding of the peptides to part only (i.e. a
subsite) of the antigen-combining surface of the antibody.

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Since the studies with synthetic peptide libraries used IgM^{Nov} serum rather than purified antibody, signals emanating from other serum IgM antibodies may have been attributed erroneously to IgM^{Nov}, or perhaps to specific

5   binding of the anti-mu-chain conjugate to peptide motifs in the libraries. In order to demonstrate the specificity of signals obtained from the synthetic libraries, two types of control experiment were performed. First, a reagent blank was used, omitting the Nov serum. Under

10   these conditions, no signal was generated. Secondly, serum was preabsorbed with Colominic acid in solution phase and the serum used to probe libraries. Preabsorption with Colominic acid resulted in the abolition or near-abolition of strong signals, leaving numerous weak signals

15   uninhibited. In this way it was possible to discern which signals were genuinely attributable to the anti-group-B polysaccharide antibody (IgM^{Nov}).

We have demonstrated that the preferred polysaccharide mimetic peptide motifs of IgM^{Nov}, with respect to linear

20   peptide sequences, are MDRYH [SEQ ID NO:33] and MDHYH [SEQ ID NO:34]. However, these data give no indication whether the motif or related motifs are peculiar to IgM^{Nov} or whether they represent a more-general characteristic of anti-group-B polysaccharide antibodies in general. We,

25   therefore, tested other paraproteins which had been identified by us previously by random screening of paraprotein containing sera as reactive with the group-B polysaccharide. Three such sera were tested against the 19-member DXYH [SEQ ID NO:32] set of motifs recognised by

30   IgM^{Nov} compared to two control sera that contained paraproteins which were not reactive with Colominic acid.

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Each of the three Colominic acid reactive paraproteins
were found to react with several members of the 19 member
DXYH [SEQ ID NO:32] family of peptides, whereas the
control paraproteins did not react with any of the 19

5   peptides. These data demonstrate that the DXYH [SEQ ID
NO:32] motif is not unique to IgM^{Nov} but represents a
more-general property of human anti-group-B polysaccharide
antibodies.

These relatively small motifs identified from the linear

10   peptide libraries may indicate that small linear peptides
of the specified sequences are adequate to occupy the
antigen-combing site of anti-group-B polysaccharide
antibodies fully. Alternatively, they may indicate that
the flexibility of linear peptides does not allow the

15   techniques used to specify structural attributes of a
larger antigenic structure, because appropriate conformers
may be too low in abundance to allow this.

We sought means to extract further structural information
from the data generated from the synthetic peptide

20   libraries. Moreover, our hypothesis and the demonstration
that IgM^{Nov} can react with such materially diverse antigens
as polysaccharide and peptides, led us to consider whether
structural inferences made from our data could lead to the
identification of further chemical species (i.e.

25   non-peptide, non-saccharide) which would comprise
potential vaccine components or further define the
stereochemical attributes of a suitable antigen. A
conformational model was therefore built using
computational methods based on structure activity data for

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a series of linear peptides reactive with IgM^{Nov}, as
follows.

Linear peptides convey little structural information.
However, we postulated that by identifying consensus

5   structural features of families of peptide ligands that
bind to IgM^{Nov}, taking into account their relative binding
activity, it should be possible to identify the
epitomising structural features of the antigenic structure
most preferred by the antibody. Such procedures have been

10   used before to identify 'pharmacophores' that epitomise
the ideal structural features of drugs, and which can be
used as structural templates to search databases of
molecular structures for novel drug leads. However, such
techniques have not previously been applied to antigens or

15   antibodies, nor (to our knowledge) have peptide libraries
been used to generate pharmacophores.

A pharmacophore is a constellation of physicochemical
features (charge, hydrophobicity, etc.) defined by a map
of their relative positions and absolute distances in

20   three-dimensional space, which epitomises the structural
attributes required for a compound to have a particular
pharmacological activity. The validity of a
pharmacophoric hypothesis can be tested experimentally by
demonstrating that exemplars of that hypothesis have the

25   predicted attributes in assays of binding or biological
activity. We now describe the application of this concept
to antibody-antigen interaction. In the case of
antibodies, there is no "activity" in a strict
pharmacological sense, although the antigenic equivalent

30   of a pharmacophore would be predicted to have immunogenic

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activity *in vivo*. With this proviso, the pharmacophoric
description of the group-B polysaccharide which follows
relates to antibody-antigen binding without implied
pharmacological activity.

5   Of all possible pharmacophores that can occupy
three-dimensional space, there are two related
pharmacophore classes that are both statistically
significant (in the sense that they describe the
structure-activity relationship of the training set of
10   peptide ligands for IgM Nov with a correlation that is
statistically significant) and also predictive of
antigenic (and potentially immunogenic) ligands, of IgM^{Nov}.
These pharmacophores were generated in the following way.

Data for pharmacophore generation was generated from the
15   primary and secondary linear octameric peptide libraries
(of general formula XOXOXXXX) referred to earlier in this
document. A training set of 19 'virtual' tripeptides was
constructed from the 'OXO' region of the octameric
peptides. The peptides comprising the training set are
20   described in the table of figure 40. Conformational models
that emphasized coverage over a 20 kCal range above the
estimated global minimum were generated in Catalyst for
each tripeptide. [Smellie, A, Teig, S.L. & Tobin,P.
J.Comp.Chem. 1995 16: 171-187; Smellie, A. Kahn, S.D.,
25   Teig S.L. J. Chem. Inf. Comp. Sci 1995 35:285]. In lieu of
dissociation constant values for the tripeptide ligands,
'relative affinity' scores were generated subjectively -
based on the time and intensity of exposure of X-ray films
exposed to the luminescent membrane-bound samples.

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In order to maximise the effectiveness of pharmacophoric searching of this dataset, the feature constraints in the table below were applied.

Feature Name	Ring Aromatic	H-bond Donor	H-bond Acceptor	Negative Ionizable	Total
minimum	0	0	0	0	1
maximum	5	5	5	5	5

Inter-feature spacing was set to 1.5 Angstroms. The mapping coefficient was set to 1, in order to force topologically similar molecules to map the generated hypothesis in similar ways. All other parameters were set to the manufacturer's default values for revision 3.0 of of the software (i.e. Catalyst).

Each of these models consisted of at least three common chemical features: a Ring Aromatic, a Negative Charge and a Hydrogen Bond Acceptor or Donor feature. (The 'common' hydrogen bonding features of the two hypotheses have shared points of origin within the tolerances of these features, although their projected points differ). The features of the pharmacophores are further defined below:

A hydrogen bond acceptor feature matches the following atom types or groups of atoms which are surface accessible;
- sp or sp^2 nitrogens that have a lone pair and a charge less than or equal to zero
- sp^3 oxygens or sulphurs that have a lone pair and charge less than or equal to zero
- non-basic amines that have a lone pair

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A hydrogen bond donor feature has the same chemical
characteristics as the hydrogen bond acceptor except that
it also includes basic nitrogen. There is no exclusion of
electron-deficient pyridines and imidazoles. This feature
matches the following atom types or groups of atoms;

- non-acidic hydroxyls
- thiols
- acetylenic hydrogens
- NH moieties (except tetrazoles and trifluoromethyl
  sulfonamide hydrogens)

A negative charge feature is defined as a negative charge
not adjacent to a positive charge.

A ring aromatic feature is defined as an aromatic moiety
which may be replaced by amino acid residues having
hydrophobic character. Tyrosine may be categorised as
"uncharged polar", but it is also somewhat hydrophobic.
Thus, according to the present invention this feature
matches the following group: methionine, alanine, leucine,
isoleucine, valine, proline, phenylalanine, tryptophan and
tyrosine.

The hydrogen bond acceptor is represented by a vector
function consisting of two spheres. The smaller sphere (at
least 1.6Angstroms radius up to 2.6 Angstroms) defines the
centroid of the hydrogen bond acceptor on the ligand while
the large sphere (at least 2.2 Angstroms radius up to 2.6
Angstroms) defines the projected point of the hydrogen
bond acceptor from the receptor. These two spheres are at
least 3.0 Angstroms apart. Similarly, the hydrogen bond

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donor is represented by a two sphere vector function as
above.

The ring aromatic is represented as two equal size spheres
(at least 1.6 Angstroms radius up to 2.0 Angstoms) whose
5   centroids are 3.1 Angstroms apart. One sphere corresponds
to the position of an aromatic ring moiety and the other
to the projected point of the electron pi stacking of the
aromatic ring system.

The negative charge is represented by a sphere at least
10   1.6Angstroms radius (up to 2.6 Angstoms). The tolerances
on all distances between these features is +/- 0.5
Angstroms and the geometric angles +/- 20 Degrees.

The distances and angles are shown in figures 1 and 2.

Position 1 indicates the ring aromatic, position 2
15   indicates the first and second possible locations of the
negative charge. Position 3 indicates the location of
either the hydrogen bond donor or hydrogen bond acceptor.
In addition to these 3 key chemical features there is a
further optional feature that may enhance selectivity in
20   screening for polysaccharide mimetic ligands for IgM^{Mov}.
Position 4 indicates the position of a possible additional
hydrogen bond donor/acceptor (i.e. an optional extra
hydrogen bond donor/acceptor feature).

To better represent these pharmacophores, reference should
25   be made to figures 3 and 4.

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Once a pharmacophoric model has been developed it is
possible to evaluate the correlation between the
pharmacophore and any selected molecular
sequence/structure.

5   For pharmacophore number 1 containing three key chemical
        features figure 5 demonstrates the fit of the two
        palindromic molecules of sequence YTE and DRY; figure 7
        demonstrates the fit of the 5mer peptide of sequence
        MDRYH. For pharmacophore 2 the fit of the same molecules
10   in the alternative pharmacophoric model is shown in
        figures 6, and 8. The figures 19 and 20 show the same two
        molecules fitted to pharmacophore 1 which includes the
        optional additional fourth chemical feature of another
        hydrogen bond donor/acceptor.

15   The predictive accuracy of the pharmacophoric model of the
        antigen was evaluated by comparing the observed activity
        of peptide ligands versus that predicted by the model. The
        resulting correlation (r=0.95) demonstrated that the data
        are highly consistent with the pharmacophoric model.

20   In order to validate this hypothetical pharmacophoric
        description of antigenic ligands for IgM[Nov], the model was
        used to search structural databases of available chemicals
        (i.e. the Available Chemicals Directory). Several of the
        top ten hits were then screened by competition ELISA using
25   IgM[Nov]. Two of these compounds, Direct Red 80 and remazol
        brilliant violet were found to be antigenically active,
        causing complete inhibition of antigen binding by IgM[Nov],
        thereby validating the model. It follows that compounds
        such as remazol brilliant violet and Direct Red 80 which

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conform to this structural model are potential candidates
for use in vaccine development. Reference should now be
had to figures 11, 12 and 22 which respectively show
remazol brilliant violet dye superimposed on
5   pharmacophore 1, pharmacophore 2, and pharmacophore 1 with
the additional fourth chemical feature.

As a further confirmation of the validity of the
pharmacophoric models represented by the patterns of
pharmacophore 1 and pharmacophore 2, a structural
10 representation of the CPS-B molecule was superimposed
respectively on pharmacophore 1, pharmacophore 2 and
pharmacophore 1 including the fourth chemical feature.
The results are shown respectively in figures 13, 14 and
23. It is notable that the fit is good in each case.

15 The synthetic peptides can be expressed on the surface of
filamentous bacteriophage using standard techniques for
phage display such as those described by Perham et al
(WO92/07077) and Greenwood et al (Greenwood J., Willis A.
E. and Perham, R. N. 1991. J. Mol Biol. 220. 821-827) to
20 produce peptide ligands in the correct structural
orientation. The constructs can then be used to evaluate
the potential of the peptide ligands to mimic the capsular
polysaccharide.

The above studies on phage peptide ligands and synthetic
25 peptide ligands for IgM^{Nov} and other human anti-group-B
polysaccharide antibodies have identified peptide
antigenic mimics of the capsular polysaccharide, and have
revealed in some detail the amino-acid residues, sequences
and structures of these peptides, and the essential

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features of non-peptide non-saccharide chemical entities,
which are necessary for mimicry of the group-B
meningococcal polysaccharide. The data described so far
infer, but do not prove, that peptides (and perhaps other
5   novel chemical entities) with these properties will be
capable of eliciting anti-polysaccharide antibodies when
formulated appropriately as phage-peptide or
peptide-protein conjugates, or with some other form of
immunogenic carrier to provide T-cell epitopes.

10   To test whether the peptides identified are immunogenic as
well as antigenic for the group-B polysaccharide, phage
clones expressing the sequences TIPLWFDDEIEVMIY [SEQ ID
NO:1] (referred to as "TIP...") and GDNFESYACVDTPCS [SEQ
ID NO:2] (referred to as "GDN...") were used to immunise
15   rats, and the resulting sera were tested for
anti-polysaccharide antibodies. Groups of six rats were
immunised subcutaneously with 12.5 microgram doses of the
phage clones, at weeks zero, 2, 4, 6 and bled at
intervals. No adjuvant was used. The results of testing
20   the pre-immune and week 8 sera show that strong responses
to the polysaccharide were observed in each of the
immunised groups, particularly that group immunised with
the GDN... phage which was the better antigen when tested
for antigenicity.

25   These findings demonstrate that the peptide ligands we
have identified are immunogenic for the polysaccharide as
well as being antigenic mimics of the polysaccharide. We
have further demonstrated that antibodies in the rat sera
were specific for the polysaccharide, since preabsorption

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of the serum with solution phase polysaccharide diminished
the signal in every case.

**Further Characterisation of Phage Library Derived
Antigenic Ligands of IgM Nov**

5   i) Analysis of peptide GDN... by Site-Directed Mutagenesis
on Phage and by Residue-Substitution of Synthetic
Peptides.

The phage clone expressing peptide GDN... was subjected to
Ala-scan mutagenesis according to standard methods wherein

10   the DNA encoding particular amino-acids in the peptide was
substituted for a codon representing alanine. Phage were
propagated in E.coli and purified by three rounds of
precipitation with polyethylene glycol. The binding of
unaltered and mutated phage clones was assesed in a solid

15   phase electrochemiluminescent (ECL) assay using an Origen
analyser (Igen). Magnetic beads were coated with a
polyclonal anti-phage (anti-M13) antibody and used to
capture phage from solution. The phage-coated beads were
then exposed successively to IgM Nov, and to an anti-mu

20   chain antibody labelled with an electrochemiluminescent
tag according to manufacturers instructions (Igen). Figure
39 shows that the GDN... expressing phage clone ('GDN')
gave a concentration-dependent signal in this assay. In
these experiments all resides of the peptides, except

25   proline (at position 10) were systematically replaced by
alanine.

It is evident from these studies that there is a clear
distinction between residues which tolerate and those

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which do not tolerate substitution by alanine. Thus, the
first four residues of this peptide (GDNF) are seen to be
unimportant to antigenicity. However, each of the cysteine
residues at positions 9 and 14 (C9, C14) were found to be
5   essential to binding, demonstrating the importance of the
short disulphide loop (CVDTPC) to antigenicity. Consistent
with these observations, residues within this loop, i.e.
V10 and D11 were not tolerant to alanine substitution,
although T12 could be replaced by alanine (relatively
10   conservative substitution). Also, E5 and Y7 were found to
be essential. It is noteworthy that the acidic residues
D,E and tyrosine (Y) were found to be essential to the
binding of IgM Nov this peptide on phage, just as was the
case with the synthetic peptide libraries, providing
15   mutual confirmation of the results of these technically
diverse approaches. In these studies however, it was found
not to be possible to substitute proline for alanine.

Next, a synthetic form of the peptide GDN... was subjected
to a 'substitution net analysis' wherein all of the
20   proteinogenic amino-acids were systematically replaced at
certain positions of the peptide. The results are
presented in the table of figure36 and refer to the region
of the GDN... peptide beginning at E5 such that the
peptide now reads ESYACVDTPCS, with E now referred to as
25   E1. The results of this study are in broad agreement with
the phage mutagenesis studies described above. Precise
agreement between these two diverse techniques (phage
disply versus synthetic peptides on membranes) would not
be expected, given the differing contexts of presentation
30   of the peptide in these two systems, and the likely
influence of context upon conformation of the peptide.

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Using the synthetic peptide approach, substitution of the
single proline residue of this peptide was accomplished
without difficulty. With the exception of serine, no other
residue was well tolerated in place of proline,
5   demonstrating the importance of this residue to the
antigenic structure of the peptide. These data also
demonstrate strikingly that D11 is important for binding,
all substitutions except alanine not being tolerated. V10
was intolerant to alanine substitution by this method,
10   although numerous other residues could be tolerated at
this position. Although Y7 was found to be tolerant (by
this method) to alanine substitution, its importance for
antigenicity was confirmed by the finding that virtually
all other substitutions attenuated or abolished binding.

15   According to this latter analysis, using peptides
synthesized on membranes, certain residues were suggested
to be preferred over the original sequence. These were D
in place of E at position 1 of this 12-mer, giving
DSYACVDTPCS. Also, at position 2, A was preferred over the
20   original S giving EAYACVDTPCS.

ii) Analysis of peptide TIP... by Residue-Substitution of
Synthetic Peptides.

Peptide TIP... was subjected first to a 'window-scan'
analysis whereby peptides were made on membrane (as above)
25   representing subsequences comprising residues 1-8, 2-9, 3-
10 etc.of this 15mer peptide. This analysis (not shown)
identified two overlapping peptides of sequence DDEIEVMI
and DEIEVMIY respectively to be antigenically active,
localising the antigenicity of the 15mer peptide TIP... to

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a smaller 9-mer sequence (i.e. DDEIEVMIY). To ascertain
precisely which residues of this peptide were most
important for antigenic mimicry of the polysaccharide, a
replacement net was constructed wherein each of the 9
residues of the sequence DDEIEVMIY were replaced by 9 of
the proteinogenic amino acids (namely
A,D,E,H,K,N,P,S,V,W,Y). The results are summarised in the
table of figure 37.

It was found that most of these residues (i.e. all except
D1) were highly intolerant to substitution, whereas the
first residue (D1) could be replaced by any of several
amino acids of diverse character, Thus, although the
acidic and hydrophillic residue 'E' (similar to the
original D) was preferred over D at the D1 position, so
too was valine (a hydrophobic branched chain amino acid).
Even tryptophan (a large aromatic hydrophobe, very
different in character to the original D) was well
tolerated at this position. The most antigenic part of
peptide TIP can be seen from this analysis to be
'DEIEVMIY'. It is notable that this sequence contains both
tyrosine and the acidic residues D and E as important
features, as for the peptides identified by the synthetic
library studies and as in the case of the GDN... peptide
(above). Thus, the substitution net of the 15mer phage-
derived peptide TIP... demonstrates the existence of a
core region which is highly optimised for antibody
binding, namely DEIEVMIY, being surprisingly intolerant of
substitution. Most notably this peptide is richly provided
with residues capable of fulfilling the requirements of
the 'combined pharmacophore' description of the epitope
(i.e. hydrophobic/aromatic and acidic residues). It is

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likely therefore that various conformations or aspects of this peptide can mimic the polysaccharide epitope. This may explain why this peptide was selected during phage display as a favoured antigenic sequence by the antibody.

5   As was the case with the GDN... peptide above, it was found in this analysis of the TIP... peptide to be possible to enhance the antigenicity of the original peptide. Thus, the non-conservative substitution of E3 with proline (P an uncharged cyclic *imino* acid) to give

10   the sequence DDPIEVMIY gave rise to an increase in reactivity with the anti-polysaccharide antibody. Also, replacement of the penultimate I (I8) with Y was found to increase antigenicity modestly. Although such a modest increase may not have been significant, this finding

15   demonstrates at least that tyrosine is tolerated at this position.

Generation of a 'Combined Pharmacophore'

The two alternative pharmcophores were found to be identical except for the positioning of a negative

20   ionizable feature. According to their descriptions, the negative ionizable feature could be in one of two alternative positions. However, another interpretation of these findings is that the antigen-binding requirements of the antibody could be satisfied better by a molecule which

25   would provide the common features of these two hypotheses, but in addition, simultaneously satisfying *both* of the negative ionizable features suggested originally to be alternatives.

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In order to avoid generating a combined hypothesis which was too complex to search or too computationally laborious to generate, the optional hydrogen bond donor feature (common to both hypotheses) was discarded. The two

5   constituent hypotheses were superposed using the root-mean square fitting algorithm of Catalyst to generate the combined pharmacophore. Thus, the common ring aromatic feature, the two negative ionizables (originating separately from the different constituent pharmacophores),

10   and the H-bond donor and acceptor features (having differing vectors - yet a common centroid point of origin) were combined to generate a new 5-feature hypothesis. The combined hypothesis was used to search the Available Chemicals Directory, resulting in only 2 hits, one of

15   which transpired to be the most active compound yet identified (i.e. methylmalonyl coenzyme-A at an IC50 of 7μM).

In order to increase the catchment of the combined pharmacophore to allow the identification of further

20   active compounds, the tolerance on each of the two negative ionizable features of the 5-feature combined pharmacophore was increased by 1 Angstrom. This resulted in recovery of 11 hits from the ACD, illustrated in the table of figure 40. The results of searching the ACD with

25   the various hypotheses is summarized below.

From a database search of the ACD, 26 and 171 compounds were recovered as 'hits' (i.e. mapping all features of the pharmacophoric hypothesis) for the two alternative constituent pharmacohphores, respectively, when searched

30   independently. These compounds were subject to a further

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screen to avoid duplication and to eliminate potentially
reactive species, a representative sample set of these
compounds was tested by immunoassay. With hypothesis 1, of
9 compounds procured and tested, 6 were antigenically
active (67%). Similarly, with hypothesis 2, all 12
compounds procured were found to be antigenically active.
(i.e. IC50's less than 1mM).

We tested several of the compounds retrieved by the
combined pharmacophore (with expanded negative ionizable
features - see above), and found a surprisingly high
fraction of them to be antigenically active. Thus, of 11
compounds identified, 8 were procured and 4 of these were
strongly antigenically active (IC50 < 200μM), the
remaining ones weakly so (>410 μM). The strongly active
compounds were: nicotinamide 1, N6 ethanoadenine
dinucleotide phosphate, P1,P5-di (adenosine-5')
pentaphosphate, nicotinamide hypoxanthine dinucleotide
phopshate and methylmalonyl coenzyme A (IC50 7μM). This is
a remarkably high hit rate (50%) compared to the rates
expected for random screening of compounds (<1%) and
therefore represents strong evidence for the validity of
the more-detailed 'combined' form of the pharmacophoric
hypothesis. Notably, the non-phosphorylated form of N6
ethenoadenine dinucleotide phosphate was markedly less
active (IC50 of 150μM) than its phosphorylaed counterpart
(IC50, 11μM) emphasizing the importance of having two
negative charges.

**Relative Importance of Negative Ionizables Versus Other
Features of the 'Combined Pharmacophore'**

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In order to assess whether the posession of a pair of
appropriately spaced negative ionizables was pre-eminently
important to antigenicity, or whether other aspects of the
pharmacophoric description comprising the 'combined
5   pharmacophore' were comparbly important, an investigation
was carried out using the di-adenosine polyphosphate
series of compounds. A search with the combined
pharmacophore and immunosassay had identified P1', P5'
diadenosine polyphosphate as an antigenically active
10   compound. Advantage was taken of the existence of an
homologous series of these compounds ranging from the
triphosphate to the hexaphosphate. Figure 38 illustrates a
comparison of the antigenic potency of this family of
compounds in a hapten inhibition assay (the assay used to
15   determine IC50 values for haptenic antigens). It is
evident from this figure that although all of the
compounds (i.e. tri- up to hexa-phosphate) were fully
antigenically active, there was a clear gradation of
potency - increasing up to the pentaphosphate and being
20   increased only marginally on moving up to the
hexaphosphate homologue.

These observations with the diadenosine polyphosphates
emphasize that although the two negative ionizable
features of the hypothesis are important, yet other
25   features of the hypothesis are also important. Thus, in
ascending this homologous series, the pentaphosphate is
the first compound capable of spanning the two negative
ionizable features. Nevertheless, the tri and tetra
phosphates were also active, although less so, despite
30   being unable to satisfy simultaneously both negative
ionizable features. The fact that these latter compounds

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were active demonstrates that the satisfaction of one only

of the two negative ionizable features is sufficient for

antigenicity, provided that the other features of the

hypothesis are satisfied. (Incidentally, it appears that

5   the ring aromatic and hydrogen bonding features of the

pharmacophore are satisfied in this family of compounds by

the adenine moiety, and not as intuition might suggest -

by the ribose emulating the saccharide moieties of the

polysaccharide).

10   It is also evident from the figure depicting the

comparative potency of the diadenosine polyphosphate

series (Figure 38) that these compounds are highly

specific for the antibody IgM_{Nov}. In contrast, the

polysaccharide binding of antibody 64V (an isotype matched

15   human paraprotein antibody also recognizing the group-B

meningococcal polysaccharide) was not inhibited at all by

these compounds in the range tested.

A striking feature of the small molecules identified by

the combined pharmacophore was that their 'affinities'

20   (i.e. IC50's) approximate that of the polysaccharide

itself (i.e. IC50 for colominic acid was found to be

1.8μM). This is remarkable given the fact that the sialic

acid monomer, of which this poymer is comprised, was found

to be completely inactive in these assays (i.e. IC50 >>

25   1.0 mM). It is also remarkable given the valency advantage

of colominic acid as an inhibitor, relative to the

monovalent haptenic ligands identified from pharmacophore

searching. Thus, with a degree of polymerisation of n=30

the polymer has an avidity advantage, such that it can

- 46 -

simultaneously express the conformational epitope several times over.

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EXAMPLE 1

A recombinant filamentous bacteriophage (fd) clone
expressing the sequence GDNFESYACVDTPCS [SEQ ID NO:2]
(GDN...) fused to the N-terminal of the gene-VIII protein

5   was purified by caesium chloride gradient sedimentation
and injected subcutaneously in 12.5 microgram doses into
six Wistar rats. This procedure was repeated for each of
the six rats a further two times at 2-week intervals, and
serum samples were taken at intervals. In order to test

10   preimmune and week 8 post-immunisation sera for
reactivity with colominic acid (alpha 2-8 linked
polysialic acid, the capsular polysaccharide of *E. coli* K1
and the group-B meningococci), polystyrene ELISA plates
coated with colominic acid were incubated first with

15   serial dilutions of the sera and secondly with a
species-specific anti-IgG alkaline-phosphatase conjugate,
and finally developed with a chromogenic substrate for
alkaline phosphatase. There was a highly significant
increase in the assay signal (absorbance) in the

20   post-immunization bleed, demonstrating the elicitation of
IgG class antibodies against the polysaccharide. The IgG
class of the antibodies is particularly significant, since
it demonstrates 'class-switching' of the immune response,
a hallmark of immunological memory. It is also significant

25   that this response was obtained in the absence of
adjuvant, since it demonstrates that the phage construct
is particularly immunogenic for this notoriously weak
antigen (i.e. the group-B polysaccharide).

Spurious signals can be generated in ELISA tests via the

30   non-specific binding of antibodies to solid phase

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components other than the antigen (e.g. the polystyrene
itself or the blocking agent used to minimise non-specific
binding to the polystyrene). These signals may be
distinguished from specific signals, because only the
5   latter are inhibited by preabsorption with colominic acid
in solution phase. We have demonstrated that the binding
of phage-peptide elicited antibodies by clone GDN... can
be inhibited substantially by solution phase colominic
acid, verifying the specificity of these phage-peptide
10   elicited antibodies, and confirming their immunogenicity
for the polysaccharide.

EXAMPLE 2

A second phage clone TIPLWFDDEIEVMIY [SEQ ID NO:1]
(TIP...) was also used to immunise rats, precisely as
15   described above (EXAMPLE 1). We have demonstrated that
this phage clone also elicited anti-polysaccharide
antibodies which were inhibited by solution phase
polysaccharide.

EXAMPLE 3

20   A further peptide ligand according to the inventurn is
GECPTGSLLLDGLYC [SEQ ID NO: 38]. This peptide ligand has
tyrosine (Y) and glutamic acid (E) residues brought into
proximity by means of a disulphide bond.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT:

        (A) NAME: PEPTIDE THERAPEUTICS LIMITED

        (B) STREET: 321 CAMBRIDGE SCIENCE PARK

        (C) CITY: CAMBRIDGE

        (D) STATE: CAMBRIDGE

        (E) COUNTRY: ENGLAND

        (F) POSTAL CODE (ZIP): CB4 4WG

        (G) TELEPHONE: 01223 423333

        (H) TELEFAX: 01223 423111

    (ii) TITLE OF INVENTION: Meningococcal Vaccine

    (iii) NUMBER OF SEQUENCES: 38

    (iv) COMPUTER READABLE FORM:

        (A) MEDIUM TYPE: Floppy disk

        (B) COMPUTER: IBM PC compatible

        (C) OPERATING SYSTEM: PC-DOS/MS-DOS

        (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(EPO)

    (2) INFORMATION FOR SEQ ID NO: 1:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 15 amino acids

        (B) TYPE: amino acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Thr Ile Pro Leu Trp Phe Asp Asp Glu Ile Glu Val Met Ile Tyr
1   5   10   15

(2) INFORMATION FOR SEQ ID NO: 2:

5   (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 15 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

10   (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Gly Asp Asn Phe Glu Ser Tyr Ala Cys Val Asp Thr Pro Cys Ser
1   5   10   15

(2) INFORMATION FOR SEQ ID NO: 3:

15   (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 8 amino acids
                    (B) TYPE: amino acid
                    (C) STRANDEDNESS: single
                    (D) TOPOLOGY: linear

20   (ii) MOLECULE TYPE: peptide

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        (ix)  FEATURE:

              (A)  NAME/KEY: Modified-site

              (B)  LOCATION:2

              (D)  OTHER INFORMATION:/note= "identity of residue at
 5      this position is known"


        (ix)  FEATURE:

              (A)  NAME/KEY: Modified-site

              (B)  LOCATION:4

              (D)  OTHER INFORMATION:/note= "identity of residue at
10      this position is known"



        (xi)  SEQUENCE DESCRIPTION: SEQ ID NO: 3:


        Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        1                   5


    (2)  INFORMATION FOR SEQ ID NO: 4:


15       (i)  SEQUENCE CHARACTERISTICS:

              (A)  LENGTH: 8 amino acids

              (B)  TYPE: amino acid

              (C)  STRANDEDNESS: single

              (D)  TOPOLOGY: linear


20       (ii)  MOLECULE TYPE: peptide




        (xi)  SEQUENCE DESCRIPTION: SEQ ID NO: 4:


        Xaa Ala Xaa Ala Xaa Xaa Xaa Xaa
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1   5

(2) INFORMATION FOR SEQ ID NO: 5:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 8 amino acids

5   (B) TYPE: amino acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

10   Xaa Ala Xaa Asp Xaa Xaa Xaa Xaa

    1   5

(2) INFORMATION FOR SEQ ID NO: 6:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 8 amino acids

15   (B) TYPE: amino acid

        (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Xaa Tyr Xaa Glu Xaa Xaa Xaa Xaa
1   5

(2) INFORMATION FOR SEQ ID NO: 7:

        (i) SEQUENCE CHARACTERISTICS:
5   (A) LENGTH: 8 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

10   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

        Xaa Asp Xaa Tyr Xaa Xaa Xaa Xaa
        1   5

    (2) INFORMATION FOR SEQ ID NO: 8:

        (i) SEQUENCE CHARACTERISTICS:
15   (A) LENGTH: 8 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

20   (ix) FEATURE:
                (A) NAME/KEY: Modified-site
                (B) LOCATION:1

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            (D) OTHER INFORMATION:/note= "identity of residue at
      this position is known"


            (ix) FEATURE:
                 (A) NAME/KEY: Modified-site
5                (B) LOCATION:2
                 (D) OTHER INFORMATION:/note= "identity of residue at
      this position is known"



            (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:


            Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
10          1                 5


      (2) INFORMATION FOR SEQ ID NO: 9:


            (i) SEQUENCE CHARACTERISTICS:
                 (A) LENGTH: 8 amino acids
                 (B) TYPE: amino acid
15               (C) STRANDEDNESS: single
                 (D) TOPOLOGY: linear


            (ii) MOLECULE TYPE: peptide



            (ix) FEATURE:
                 (A) NAME/KEY: Modified-site
20               (B) LOCATION:1
                 (D) OTHER INFORMATION:/note= "identity of residue at
      this position is known"


            (ix) FEATURE:
                 (A) NAME/KEY: Modified-site
25               (B) LOCATION:3
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(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1   5

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION:2

(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION:5

(D) OTHER INFORMATION:/note= "identity of residue at this position is known"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1   5

(2) INFORMATION FOR SEQ ID NO: 11:

        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 8 amino acids
                (B) TYPE: amino acid
                (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (ix) FEATURE:
                (A) NAME/KEY: Modified-site
                (B) LOCATION:4
                (D) OTHER INFORMATION:/note= "identity of residue at
this position is known"

        (ix) FEATURE:
                (A) NAME/KEY: Modified-site
                (B) LOCATION:7
                (D) OTHER INFORMATION:/note= "identity of residue at
this position is known"

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

        Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        1   5

(2) INFORMATION FOR SEQ ID NO: 12:

        (i) SEQUENCE CHARACTERISTICS:

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       (A) LENGTH: 8 amino acids

       (B) TYPE: amino acid

       (C) STRANDEDNESS: single

       (D) TOPOLOGY: linear

5   (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

     Arg Glu Xaa Xaa Xaa Xaa Xaa Xaa
     1   5

(2) INFORMATION FOR SEQ ID NO: 13:

10   (i) SEQUENCE CHARACTERISTICS:

       (A) LENGTH: 8 amino acids

       (B) TYPE: amino acid

       (C) STRANDEDNESS: single

       (D) TOPOLOGY: linear

15   (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

     Thr Asp Xaa Xaa Xaa Xaa Xaa Xaa
     1   5

(2) INFORMATION FOR SEQ ID NO: 14:

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```
              (i) SEQUENCE CHARACTERISTICS:

                   (A) LENGTH: 8 amino acids

                   (B) TYPE: amino acid

                   (C) STRANDEDNESS: single

 5                 (D) TOPOLOGY: linear


             (ii) MOLECULE TYPE: peptide




             (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:


             Thr Glu Xaa Xaa Xaa Xaa Xaa Xaa
             1                   5


10      (2) INFORMATION FOR SEQ ID NO: 15:


              (i) SEQUENCE CHARACTERISTICS:

                   (A) LENGTH: 8 amino acids

                   (B) TYPE: amino acid

                   (C) STRANDEDNESS: single

15                 (D) TOPOLOGY: linear


             (ii) MOLECULE TYPE: peptide




             (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:


             His Xaa Asp Xaa Xaa Xaa Xaa Xaa
             1                   5


20      (2) INFORMATION FOR SEQ ID NO: 16:
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     (i) SEQUENCE CHARACTERISTICS:

          (A) LENGTH: 8 amino acids

          (B) TYPE: amino acid

          (C) STRANDEDNESS: single

5   (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

     Asp Xaa His Xaa Xaa Xaa Xaa Xaa
     1   5

10   (2) INFORMATION FOR SEQ ID NO: 17:

     (i) SEQUENCE CHARACTERISTICS:

          (A) LENGTH: 8 amino acids

          (B) TYPE: amino acid

          (C) STRANDEDNESS: single

15   (D) TOPOLOGY: linear

     (ii) MOLECULE TYPE: peptide

     (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

     Leu Xaa His Xaa Xaa Xaa Xaa Xaa
     1   5

20   (2) INFORMATION FOR SEQ ID NO: 18:

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```
        (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 8 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear


       (ii) MOLECULE TYPE: peptide




       (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:


       Xaa Asp Xaa His Xaa Xaa Xaa Xaa
       1                   5


    (2) INFORMATION FOR SEQ ID NO: 19:


        (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 8 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear


       (ii) MOLECULE TYPE: peptide




       (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:


       Xaa Xaa Asp His Xaa Xaa Xaa Xaa
       1                   5


    (2) INFORMATION FOR SEQ ID NO: 20:
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```
        (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 8 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
  5           (D) TOPOLOGY: linear


       (ii) MOLECULE TYPE: peptide




       (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:


       His Xaa Xaa Glu Xaa Xaa Xaa Xaa
       1               5


 10    (2) INFORMATION FOR SEQ ID NO: 21:


        (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 8 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
 15           (D) TOPOLOGY: linear


       (ii) MOLECULE TYPE: peptide




       (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:


       Xaa His Xaa Xaa Glu Xaa Xaa Xaa
       1               5


 20    (2) INFORMATION FOR SEQ ID NO: 22:
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     (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 8 amino acids

        (B) TYPE: amino acid

        (C) STRANDEDNESS: single

5   (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Xaa Asp Xaa Xaa His Xaa Xaa Xaa
1   5

10 (2) INFORMATION FOR SEQ ID NO: 23:

     (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 8 amino acids

        (B) TYPE: amino acid

        (C) STRANDEDNESS: single

15   (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Xaa Xaa His Xaa Xaa Glu Xaa Xaa
1   5

20 (2) INFORMATION FOR SEQ ID NO: 24:

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        (i) SEQUENCE CHARACTERISTICS:

             (A) LENGTH: 8 amino acids

             (B) TYPE: amino acid

             (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

    Xaa Xaa Xaa His Xaa Xaa Glu Xaa
    1   5

    (2) INFORMATION FOR SEQ ID NO: 25:

        (i) SEQUENCE CHARACTERISTICS:

             (A) LENGTH: 4 amino acids

             (B) TYPE: amino acid

             (C) STRANDEDNESS: single

        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

    His Xaa Xaa Glu
    1

    (2) INFORMATION FOR SEQ ID NO: 26:

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        (i) SEQUENCE CHARACTERISTICS:

            (A) LENGTH: 4 amino acids

            (B) TYPE: amino acid

            (C) STRANDEDNESS: single

5   (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

        Glu Xaa Xaa His
        1

10   (2) INFORMATION FOR SEQ ID NO: 27:

        (i) SEQUENCE CHARACTERISTICS:

            (A) LENGTH: 4 amino acids

            (B) TYPE: amino acid

            (C) STRANDEDNESS: single

15   (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

        Asp Xaa Xaa His
        1

20   (2) INFORMATION FOR SEQ ID NO: 28:

- 70 -

  (i) SEQUENCE CHARACTERISTICS:

    (A) LENGTH: 4 amino acids

    (B) TYPE: amino acid

    (C) STRANDEDNESS: single

5   (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: peptide

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

  His Xaa Xaa Asp

  1

10 (2) INFORMATION FOR SEQ ID NO: 29:

  (i) SEQUENCE CHARACTERISTICS:

    (A) LENGTH: 8 amino acids

    (B) TYPE: amino acid

    (C) STRANDEDNESS: single

15   (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: peptide

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

  Xaa Tyr Xaa Glu Xaa Xaa Xaa Xaa

  1   5

20 (2) INFORMATION FOR SEQ ID NO: 30:

- 71 -

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 8 amino acids

        (B) TYPE: amino acid

        (C) STRANDEDNESS: single

5   (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

    Xaa Asp Xaa Tyr Xaa Xaa Xaa Xaa

    1   5

10   (2) INFORMATION FOR SEQ ID NO: 31:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 8 amino acids

        (B) TYPE: amino acid

        (C) STRANDEDNESS: single

15   (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

    Xaa Asp Xaa Tyr His Xaa Xaa Xaa

    1   5

20   (2) INFORMATION FOR SEQ ID NO: 32:

- 72 -

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 4 amino acids

        (B) TYPE: amino acid

        (C) STRANDEDNESS: single

5   (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Asp Xaa Tyr His
1

10   (2) INFORMATION FOR SEQ ID NO: 33:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 5 amino acids

        (B) TYPE: amino acid

        (C) STRANDEDNESS: single

15   (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Met Asp Arg Tyr His
1   5

20   (2) INFORMATION FOR SEQ ID NO: 34:

- 73 -

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        (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 5 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
  5           (D) TOPOLOGY: linear


       (ii) MOLECULE TYPE: peptide




       (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:


       Met Asp His Tyr His
       1               5


 10  (2) INFORMATION FOR SEQ ID NO: 35:


        (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 9 amino acids
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
 15           (D) TOPOLOGY: linear


       (ii) MOLECULE TYPE: peptide




       (ix) FEATURE:
              (A) NAME/KEY: Modified-site
              (B) LOCATION:4
 20           (D) OTHER INFORMATION:/note= "identity of residue at
     this                 position is known"


       (ix) FEATURE:
              (A) NAME/KEY: Modified-site
```

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        (B) LOCATION:6

        (D) OTHER INFORMATION:/note= "identity of residue at
this   position is known"

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

5   Glu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys
        1   5

    (2) INFORMATION FOR SEQ ID NO: 36:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 7 amino acids
10   (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

15   Xaa Xaa Tyr Xaa Asp Xaa Xaa
        1   5

    (2) INFORMATION FOR SEQ ID NO: 37:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 7 amino acids
20   (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

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    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

    Arg Glu Tyr Glu Asp Met Met
    1   5

5   (2) INFORMATION FOR SEQ ID NO: 38:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 15 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
10   (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: peptide

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

        Gly Glu Cys Pro Thr Gly Ser Leu Leu Leu Asp Gly Leu Tyr Cys
        1   5   10   15

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Claims

1.   A pharmaceutical compound which includes a
chemical composition capable of adopting a structure
essentially equivalent to the pharmacophoric pattern of a
5   section of the capsular polysaccharide of *Neisseria
meningitidis* group B, optionally together with a
pharmaceutically acceptable carrier or excipient for use
as an anti-group B capsular polysaccharide immunogen.

2.   Peptides which include an amino acid sequence
10   capable of adopting a structure having an pharmacophoric
pattern essentially equivalent to the pharmacophoric
pattern of a section of the capsular polysaccharide of
Neisseria meningitidis group B.

3.   Antigenic peptide ligands which cross react with
15   antibodies against the capsular polysaccharide of group B
meningococci (CPS-B) which peptides include 1 or more
copies of a motif which consists of a ring aromatic
moiety, a spacer moiety, and a moiety having negative
charge.

20   4.   An antigenic peptide according to claim 3 having
two or more of said motifs.

5.   An antigenic peptide according to claim 4 wherein
said motifs repeat in an overlapping frame.

6   An antigenic peptide which includes an amino acid
25   sequence and structure essentially equivalent to the
pharmacophore defined as follows:

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the pharmacophore includes at least 3 chemical
features; a Ring Aromatic, a Negative Charge and a
Hydrogen Bond Acceptor or Donor feature; these features
being further defined as follows:

5   (1A)   the hydrogen bond acceptor feature
matches the following atom types or groups of atoms which
are surface accessible;

- sp or sp^2 nitrogens that have a lone pair and a
  charge less than or equal to zero
10 - sp^3 oxygens or sulphurs that have a lone pair and
  charge less than or equal to zero
- non-basic amines that have a lone pair;

(1B) the hydrogen bond donor feature has the same
chemical characteristics as the hydrogen bond acceptor
15 except that it also includes basic nitrogen (there is no
exclusion of electron-deficient pyridines and imidazoles);

this feature matches the following atom types or
groups of atoms;

- non-acidic hydroxyls
20 - thiols
- acetylenic hydrogens
- NH moieties (except tetrazoles and trifluoromethyl
  sulfonamide hydrogens);

(2) the negative charge feature is defined as a
25 negative charge not adjacent to a positive charge; and

(3) the ring aromatic feature is defined as an
aromatic moiety which may be replaced by amino acid
residues having hydrophobic character;

this feature matches the following group;
30   methionine, alanine, leucine, isoleucine, valine,
proline, phenylalanine, tryptophan and tyrosine; and

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(ia) the hydrog n bond acceptor is represented by
a vector function consisting of two spheres;

the smaller sphere (at least 1.6Angstroms radius
up to 2.6 Angstroms) defines the centroid of the hydrogen

5   bond acceptor on the ligand while the large sphere (at
least 2.2Angstroms radius up to 2.6 Angstroms) defines the
projected point of the hydrogen bond acceptor from the
receptor;

these two spheres are at least 3.0Angstroms apart;

10   (ib) the hydrogen bond donor is represented by a
two sphere vector function as (ia) above;

(ii) the negative charge is represented by a
sphere at least 1.6Angstroms radius (up to 2.6 Angstroms);
and

15   (iii) the ring aromatic is represented as two
equal size spheres (at least 1.6Angstroms radius up to 2.0
Angstroms) whose centroids are 3.1Angstroms apart;

one sphere corresponds to the position of an
aromatic ring moiety and the other to the projected point

20   of the electron pi stacking of the aromatic ring system;

and wherein the tolerances on all distances
between these features is +/- 0.5 Angstroms and the
geometric angles +/- 20 Degrees and said distances and
angles are shown in figure 1 or figure 2.

25   7.   An antigenic peptide according to claim 6 having
an additional fourth chemical feature which is a hydrogen
bond donor/acceptor.

8.   Antigenic peptides according to any one of claims
3 to 7 of 3 to 25 amino acids of the general formula:

30   X-B-C-D-Y

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wherein

X is any group of amino acids of length P;

D is any hydrophobic amino acid group;

C is any amino acid;

B is any negative residue;

Y is any group of amino acids of length Q; and

P and Q may be zero and P + Q is less than or equal to 22.

9.   A peptide according to any one of claims 3 to 8
having 6 to 20 amino acids.

10.   A peptide according to any one of claims 3 to 9
having 9 to 15 amino acids.

11.   A peptide according to claim 8 or 9 wherein P + Q
is less than or equal to 17.

12.   A peptide according to any one of claims 8 to 11
wherein P + Q is less than or equal to 8.

13.   A peptide according to any one of claims 8 to 12
wherein X contains His.

14.   A peptide according to any one of claims 8 to 13
wherein D is Tyr.

15.   A peptide according to any one of claims 8 to 14
wherein C is an uncharged amino acid.

16.   A peptide according to any one of claims 8 to 15
wherein C is Ser or Thr.

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17.   A peptide according to any one of claims 8 to 16
wherein C is Thr.

18.   A peptide according to any one of claims 8 to 17
wherein B is an acidic residue.

19.   A peptide according to any one of claims 8 to 18
wherein B is Asp or Glu.

20.   A peptide according to any one of claims 8 to 19
wherein B is Glu.

21.   A peptide according to any one of claims 8 to 20
wherein Y contains His.

22.   A peptide according to any one of claims 8 to 21
having the general formula
     X(containing a His)-Tyr-Thr-Glu-Y(containing a His).

23.   Antigenic peptides of 5 to 25 amino acids of
general formula:

                        X-ABCDE-Y
wherein
A is any amino acid;
E is any amino acid;
X is any group of amino acids of length P;
Y is any group of amino acids of length Q; and
P & Q may be zero and P + Q is less than or equal to 20
and B,C,D are as defined in claim 8.

24.   A peptide according to claim 23 wherein the
peptide is 6 to 20 amino acids.

25.   A peptide according to claim 23 or 24 wherein the
peptide is 9 to 15 amino acids.

26.   A peptide according to any one of claims 23 to 25
wherein B is Asp or Glu.

5   27.   A peptide according to any one of claims 23 to 26
wherein B is Asp.

28.   A peptide according to any one of claims 23 to 27
wherein A is Met.

29.   A peptide according to any one of claims 23 to 28
10   wherein E is His.

30.   A peptide according to any one of claims 23 to 29
wherein P + Q is less than or equal to 15.

31.   A peptide according to any one of claims 23 to 30
wherein P + Q is less than or equal to 10.

15   32.   A peptide according to any one of claims 23 to 31
wherein the peptide has the general formula X-Met-Asp-Arg-
Tyr-His-Y [SEQ ID NO:33].

33.   A peptide according to any preceding claim which
either alone or as a conjugate is immunogenic.

20   34.   An immunogenic ligand which comprises a synthetic
polypeptide selected from compounds of sequence SEQ ID
NO:1:

TIPLWFDDEIEVMIY

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and analogue and homologue derivatives thereof by virtue
of one or more amino acid addition, deletion,
substitution; together with terminal functional
derivatives thereof.

5   35.   An immunogenic ligand which comprises a synthetic
polypeptide selected from compounds of sequence SEQ ID
NO:2:

                     GDNFESYACVDTPCS

and analogue and homologue derivatives thereof by virtue
10   of one or more amino acid addition, deletion,
substitution; together with terminal functional
derivatives thereof.

36.   An immunogenic ligand which comprises a synthetic
polypeptide selected from compounds of sequence SEQ ID NO:
15   38:

                     GECPTGSLLLDGLYC

and analogue and homologue derivatives thereof by virtue
of one or more amino acid addition, deletion,
substitution,; together with terminal functional
20   derivatives thereof.

37.   An immunogenic ligand according to any of claims
34 to 36 wherein the synthetic polypeptide is not more
than 20 amino acids in length.

38.   An immunogenic ligand according to any of claims
25   34 to 37 wherein the synthetic polypeptide is no less than
5 amino acids in length.

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39.   An antigenic peptide which comprises one or more
of the trimeric motif sequences selected from the group
which comprises:

                    NFD; FDD; DEI; and EVM

or palandromes thereof.

40.   An antigenic peptide which comprises one or more
of the trimeric motif sequences selected from the group
which comprises:

                    DNF; ESY; and DTP

41.   An antigenic peptide which comprises one or more
of the trimeric motif sequences selected from the group
which comprises:

                    ECP; LLD; and DGL

or palandromes thereof.

42.   An antigenic peptide according to any one of
claims 39 to 41 which comprises two or more trimeric motif
sequences.

43.   An antigenic peptide according to claim 42 wherein
said trimeric motif sequences repeat in an overlapping
frame.

44.   An immunogenic ligand according to any preceding
claim which is the pentadecapeptide of SEQ ID NO:1

                    TIPLWFDDEIEVMIY

or the pentadecapeptide of SEQ ID NO: 2

                    GDNFESYACVDTPCS

or the pentadecapeptide of SEQ ID NO: 38

                    GECPTGSLLLDGLYC

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and terminal functional derivatives thereof.

45.   An immunogenic ligand according to any preceding
claim which is the pentadecapeptide of SEQ ID NO:1
                    TIPLWFDDEIEVMIY
or the pentadecapeptide of SEQ ID NO: 2
                    GDNFESYACVDTPCS
or the pentadecapeptide of SEQ ID NO: 38
                    GECPTGSLLLDGLYC
with N- or C- terminal cysteine substitution.

46.   An immunogenic ligand according to any preceding
claim in which the synthetic polypeptide is conjugated to
a carrier.

47.   An immunogenic ligand according to claim 46 in
which the carrier is a protein.

48.   An immunogenic ligand according to claim 46 or 47
in which the carrier is covalently bonded to the synthetic
polypeptide.

49.   An immunogenic ligand according to any of claims
46, 47 or 48 in which the synthetic polypeptide is linked
to a carrier selected from KLH, Diphtheria toxoid, and
Tetanus toxoid.

50.   An immunogenic ligand according to any one of
claims 46 to 49 in which the synthetic polypeptide is
linked to KLH by glutaraldehyde.

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51.   An immunogenic ligand according to any one of
claims 46 to 49 in which the synthetic polypeptide is
linked to KLH via the heterobifunctional linker MBS.

52.   An immunogenic ligand which comprises a
5   bacteriophage which encodes and is capable of expressing a
synthetic polypeptide as defined in any of claims 34 to
38.

53.   An antibody produced by an immune response to an
immunogenic ligand according to any preceding claim, which
10   antibody specifically recognises and binds the synthetic
polypeptide of the ligand and also the group-B
meningococcal Capsular Polysaccharide (CPS-B).

54.   An antibody according to claim 53 which is
monoclonal.

15   55.   An antibody according to claim 53 or 54 which is
humanised.

56.   A process for producing ligand according to any
one of claims 2 to 45 which comprises synthesising a
polypeptide as defined in any of claims 2 to 45 and
20   optionally conjugating said synthetic polypeptide to a
carrier.

57.   A process for producing an antibody according to
any of claims 53 to 55 which comprises producing an
antigenic ligand by the process of claim 18 and
25   subsequently administering said antigenic ligand in vivo

- 86 -

or in vitro so as to elicit an immune response resulting
in production of said antibody

58.   A process according to claim 57 including the
further step of isolating and purifying said antibody.

59.   A pharmaceutical composition containing as active
ingredient at least one ligand according to any of claims
2 to 52, and optionally including an adjuvant or
excipient.

60.   A pharmaceutical composition containing as active
ingredient at least one antibody according to any of
claims 53 to 55, and optionally including an adjuvant or
excipient.

61.   A pharmaceutical composition according to claim 59
or 60 for use in the treatment of infection by group-B
meningococcal bacteria.

62.   A pharmaceutical composition according to claim 59
or 60 for use in prophylactic prevention of infection by
group-B meningococcal bacteria.

63.   A method of treatment of infection with group-B
meningococcal bacteria, which comprises administering an
effective amount of a ligand as defined in any of claims 2
to 52.

64.   A method of treatment of infection with group-B
meningococcal bacteria, which comprises administering an

- 87 -

effective amount of an antibody according to any of claims
53 to 55.

65.   A method of treatment of infection with group-B
meningococcal bacteria, which comprises administering an
effective amount of a pharmaceutical composition according
to claim 59 or 60.

66.   A method of prophylaxis by prevention of infection
with group-B meningococcal bacteria, which comprises
administering an effective amount of a ligand according to
any one of claims 2 to 52.

67.   A method of prophylaxis by prevention of infection
with group-B meningococcal bacteria, which comprises
administering an effective amount of an antibody according
to any of claims 53 to 55.

68.   A method of prophylaxis by prevention of infection
with group-B meningococcal bacteria, which comprises
administering an effective amount of a pharmaceutical
composition according to claim 59 or 60.

69.   Use of a ligand according to claims 2 to 52 in the
manufacture of a medicament for the treatment of infection
with group-B meningococcal bacteria.

70.   Use of an antibody according to claims 53 to 55
in the manufacture of a medicament for the treatment of
infection with group-B meningococcal bacteria.

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71.   Use of a pharmaceutical composition according to
claim 59 or 60 in the manufacture of a medicament for the
treatment of infection with group-B meningococcal
bacteria.

5   72.   Use of a ligand according to claims 2 to 52 for
the manufacture of a medicament for prophylactic
prevention of infection with group-B meningococcal
bacteria.

73.   Use of an antibody according to claims 53 to 55
10   for the manufacture of a medicament for prophylactic
prevention of infection with group-B meningococcal
bacteria.

74.   Use of a pharmaceutical composition according to
claim 59 or 60 for the manufacture of a medicament for
15   prophylactic prevention of infection with group-B
meningococcal bacteria.

75.   An immunogenic ligand, antibody (other than known
antibody IgM^Nov) or pharmaceutical composition
substantially as hereinbefore described.
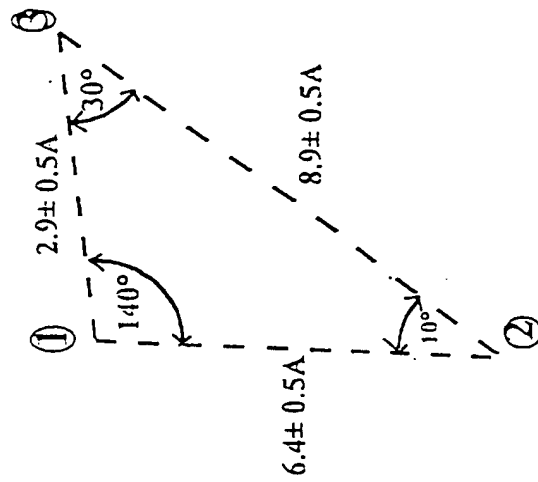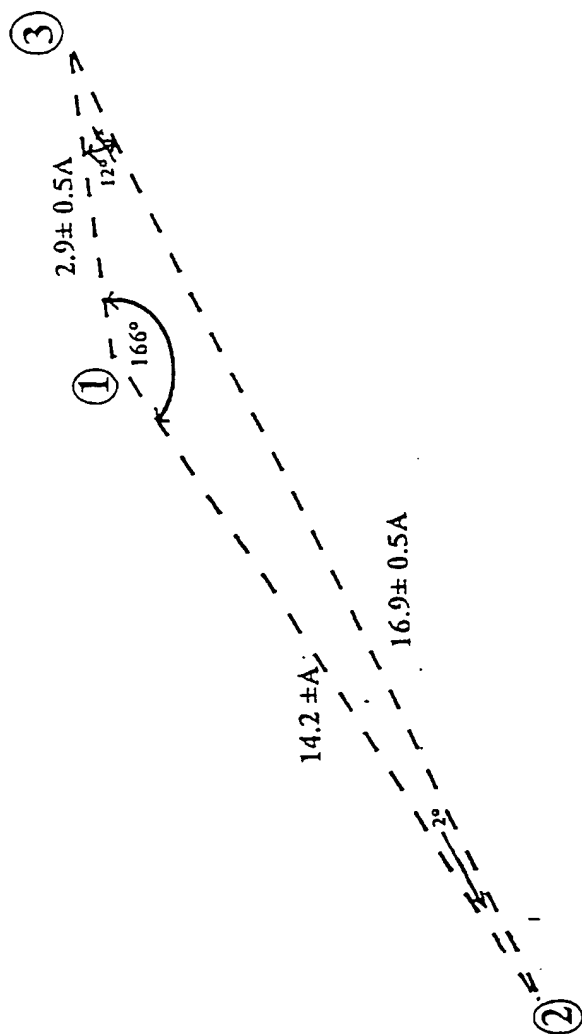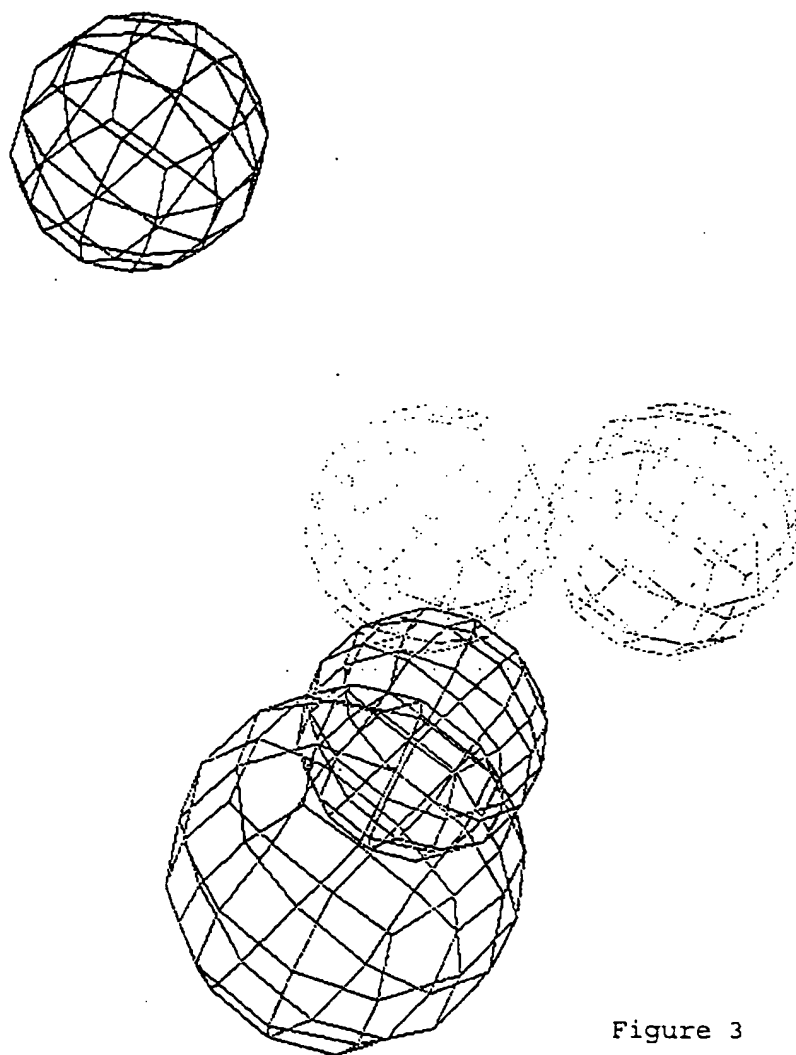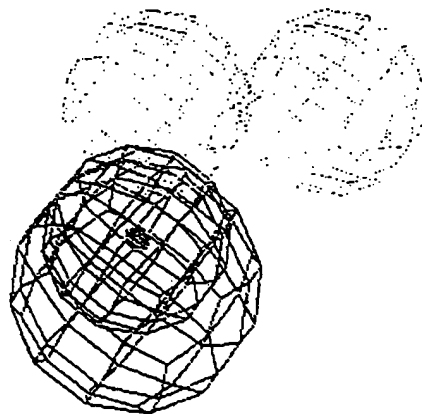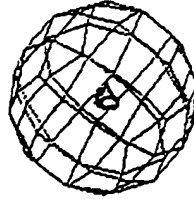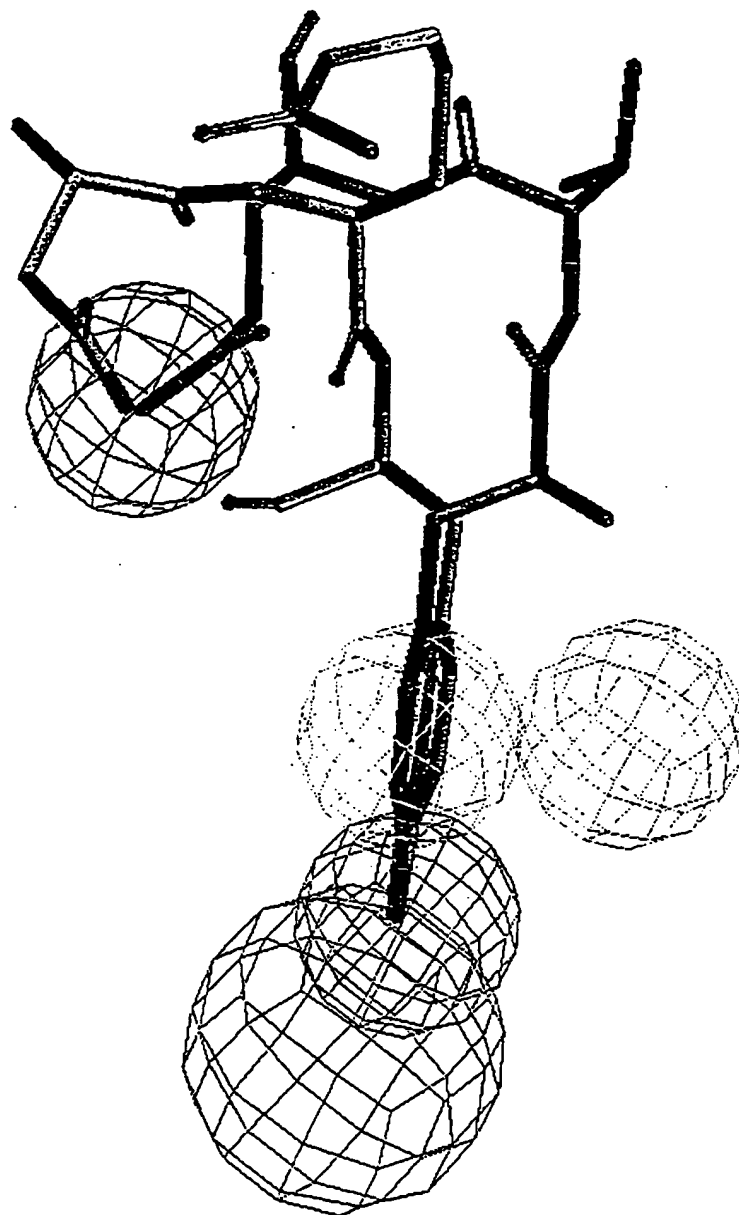
Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

8/40
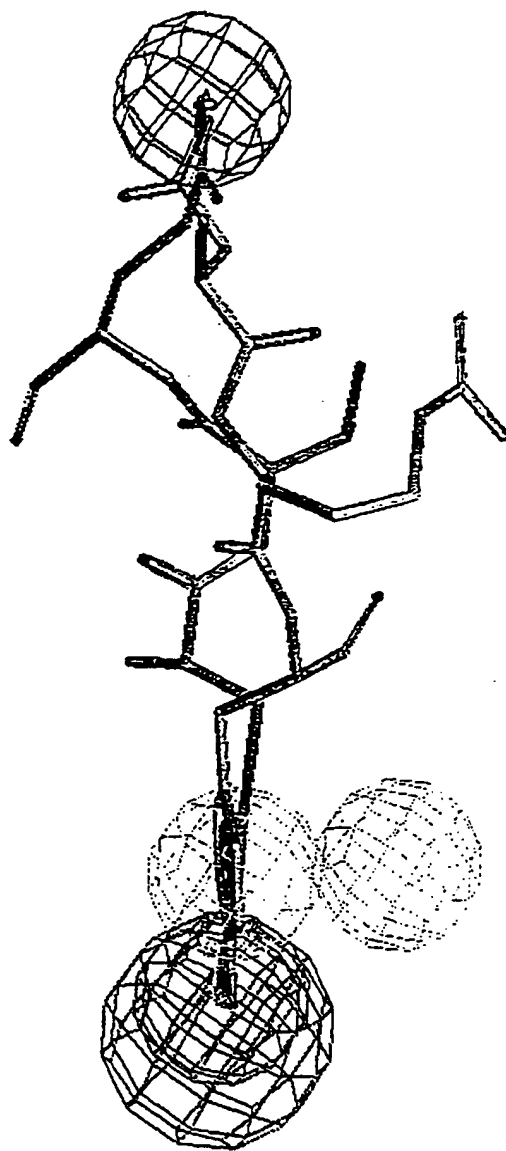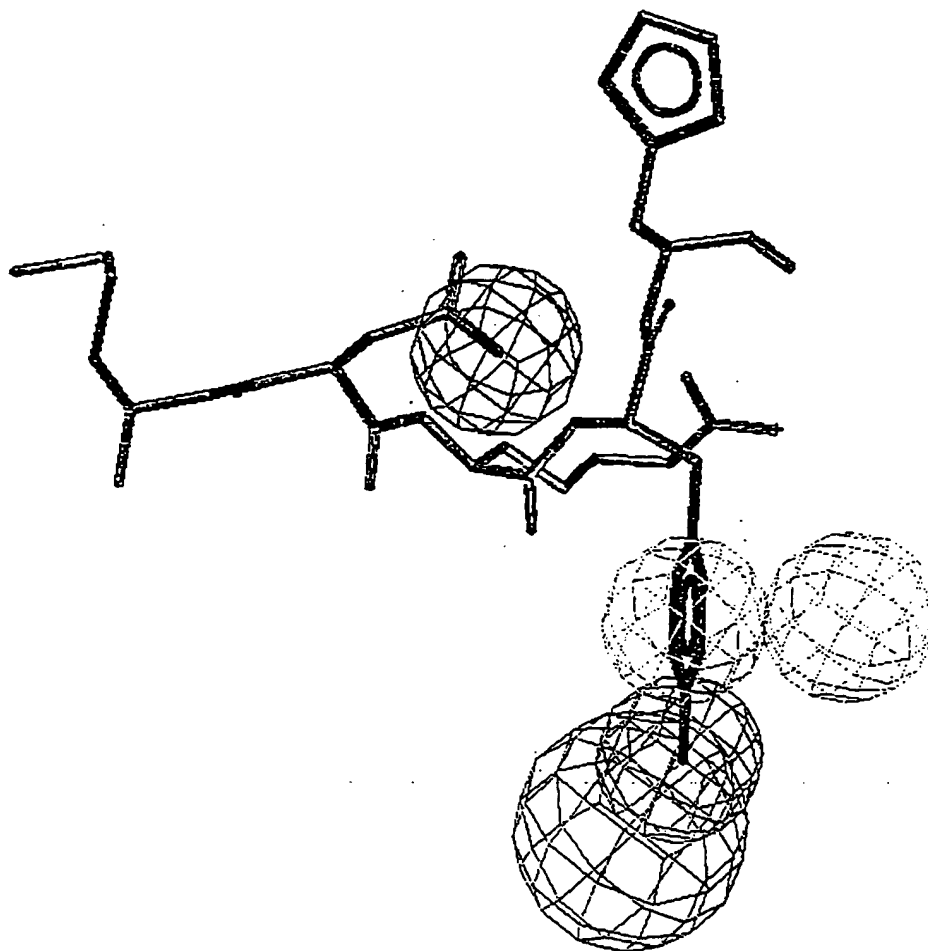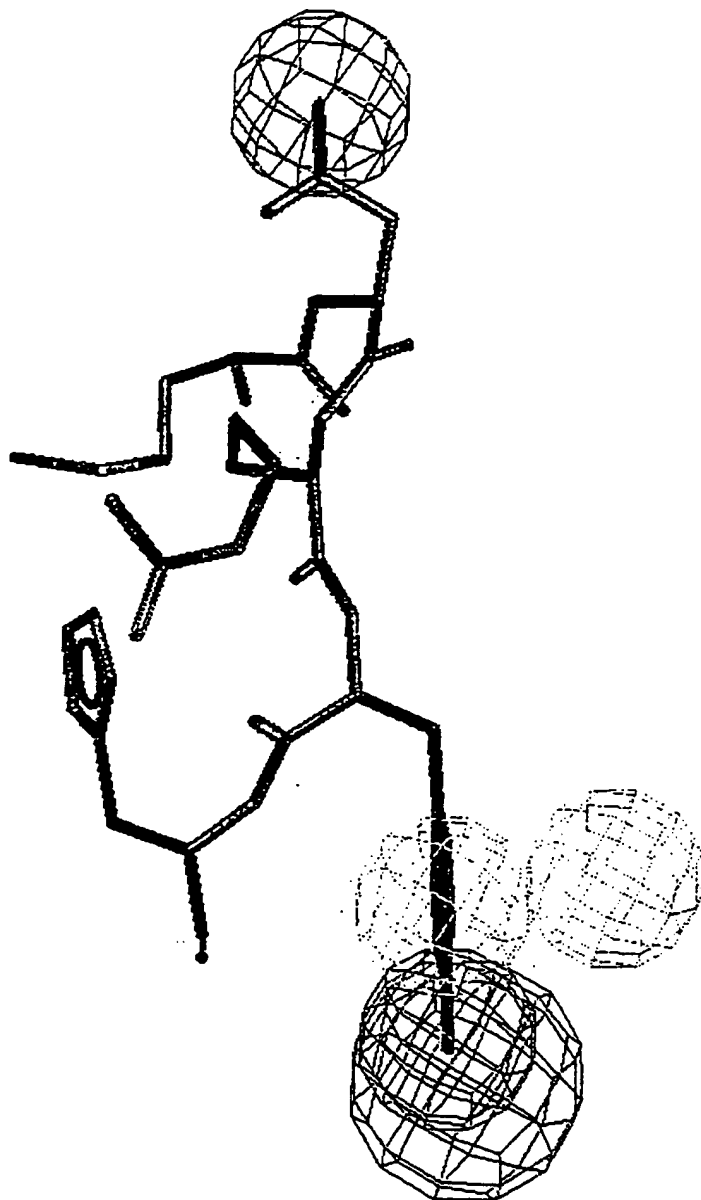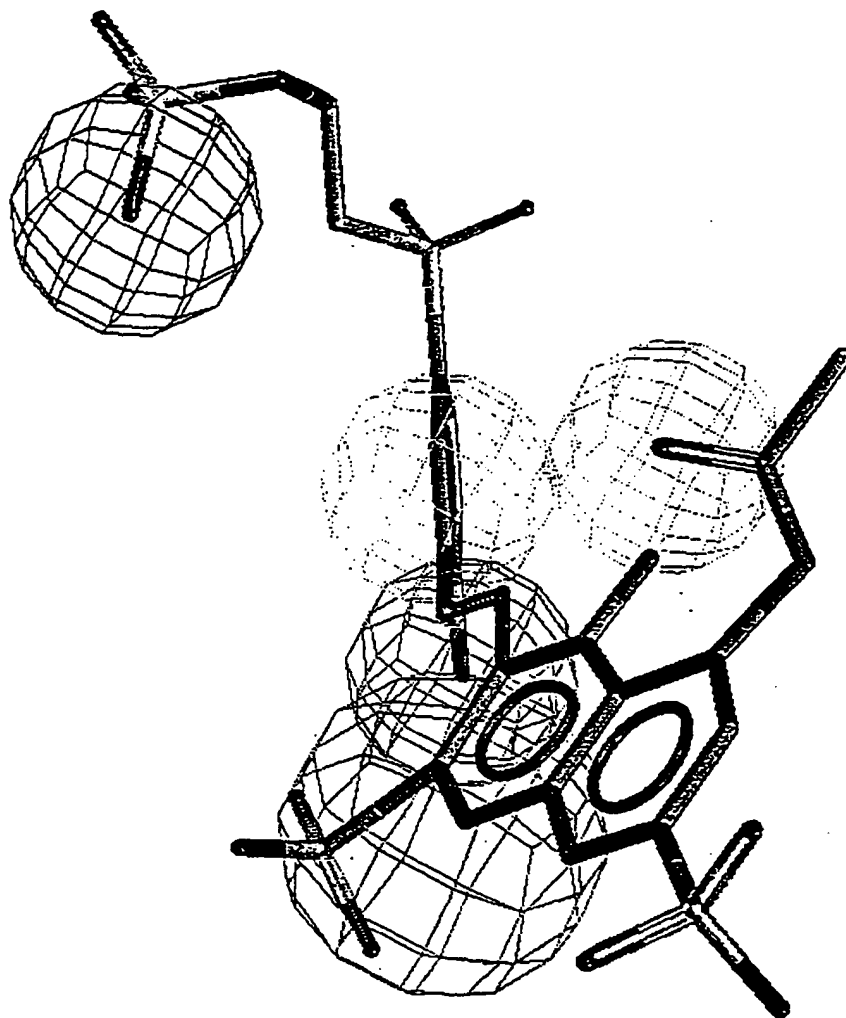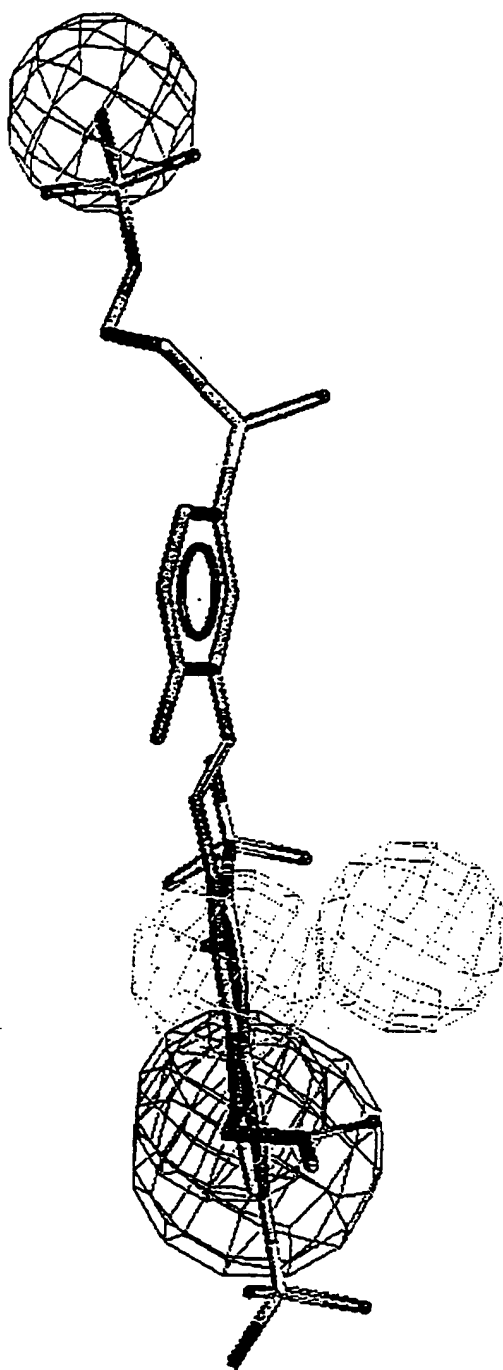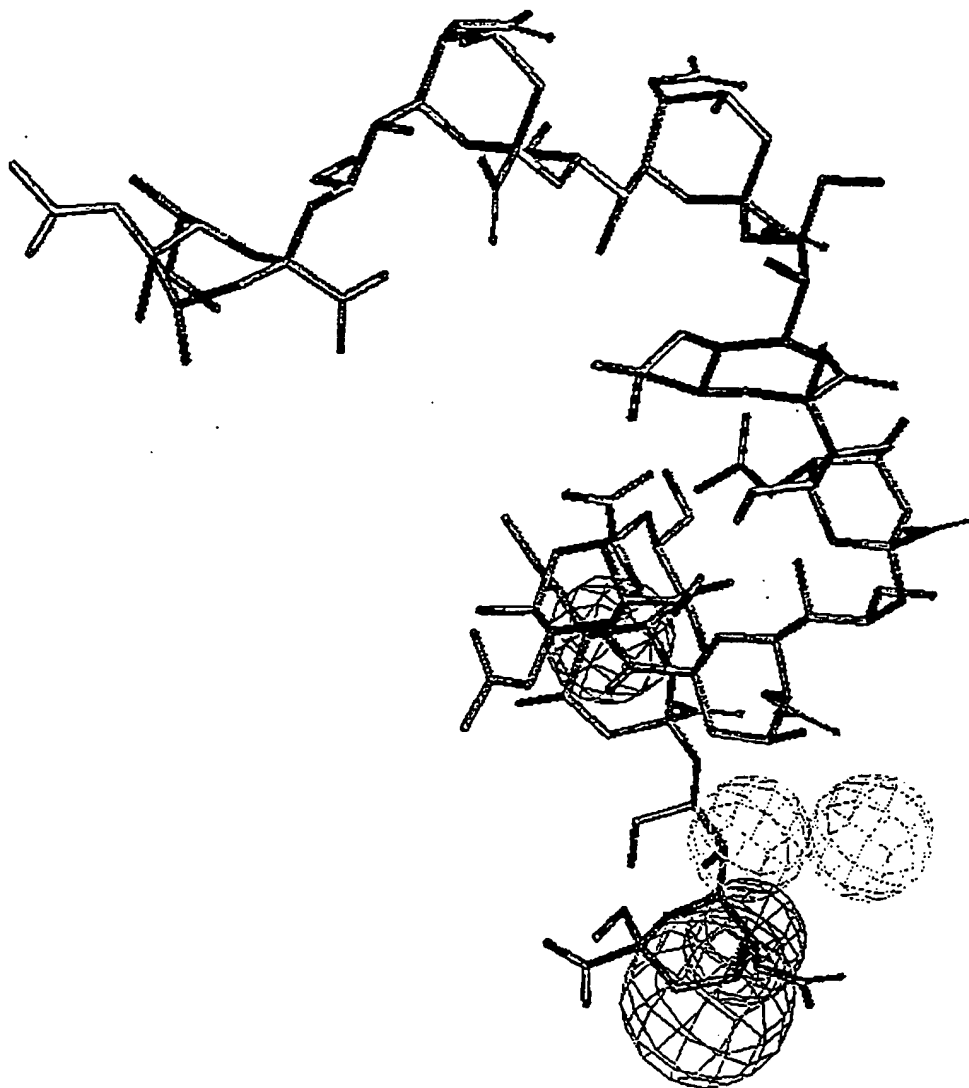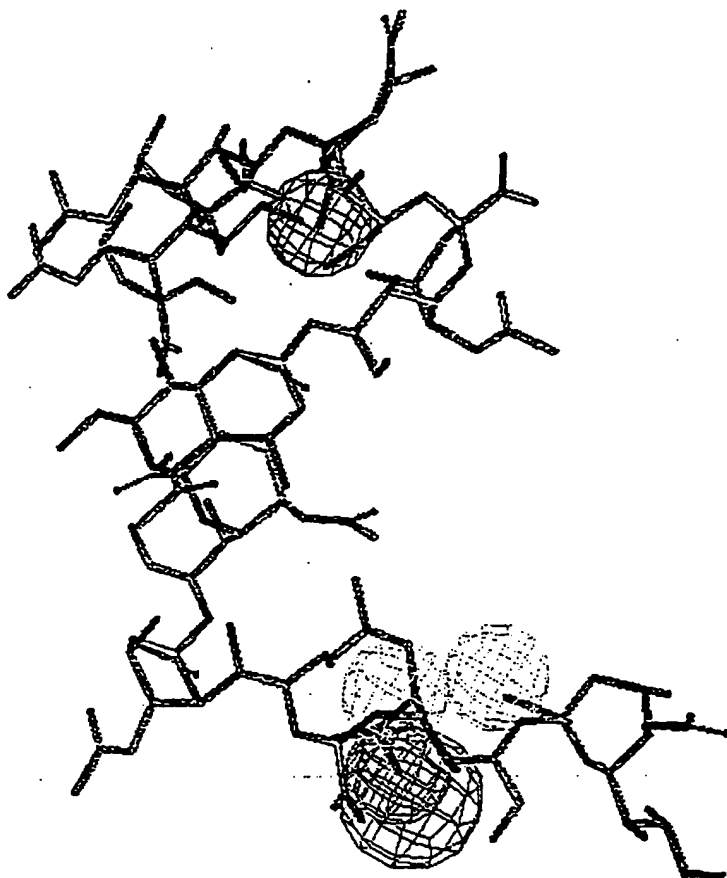
Figure 8

Figure 9

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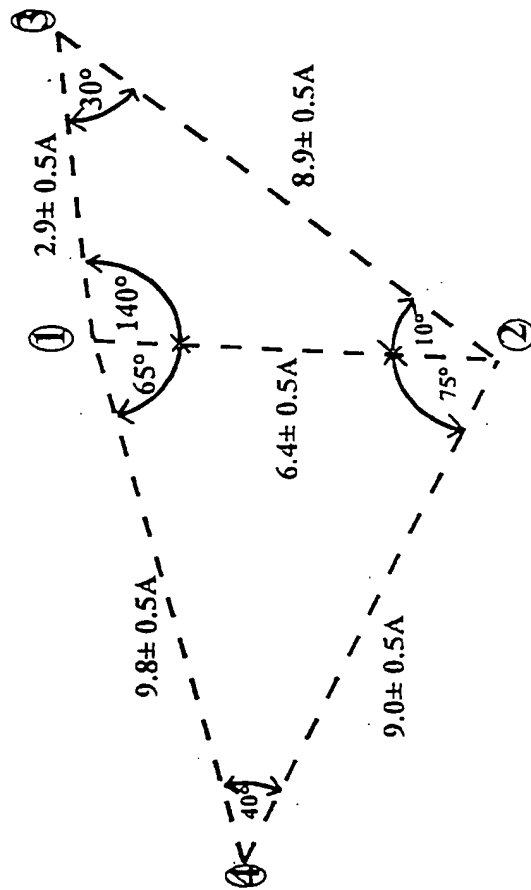
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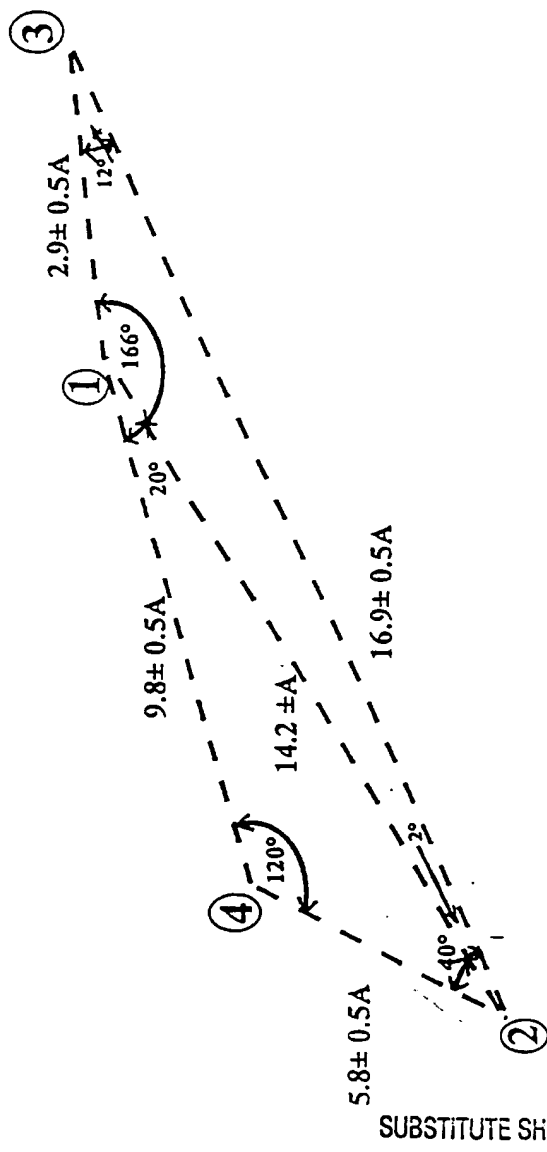
Figure 12

Figure 13

Figure 14

15/40
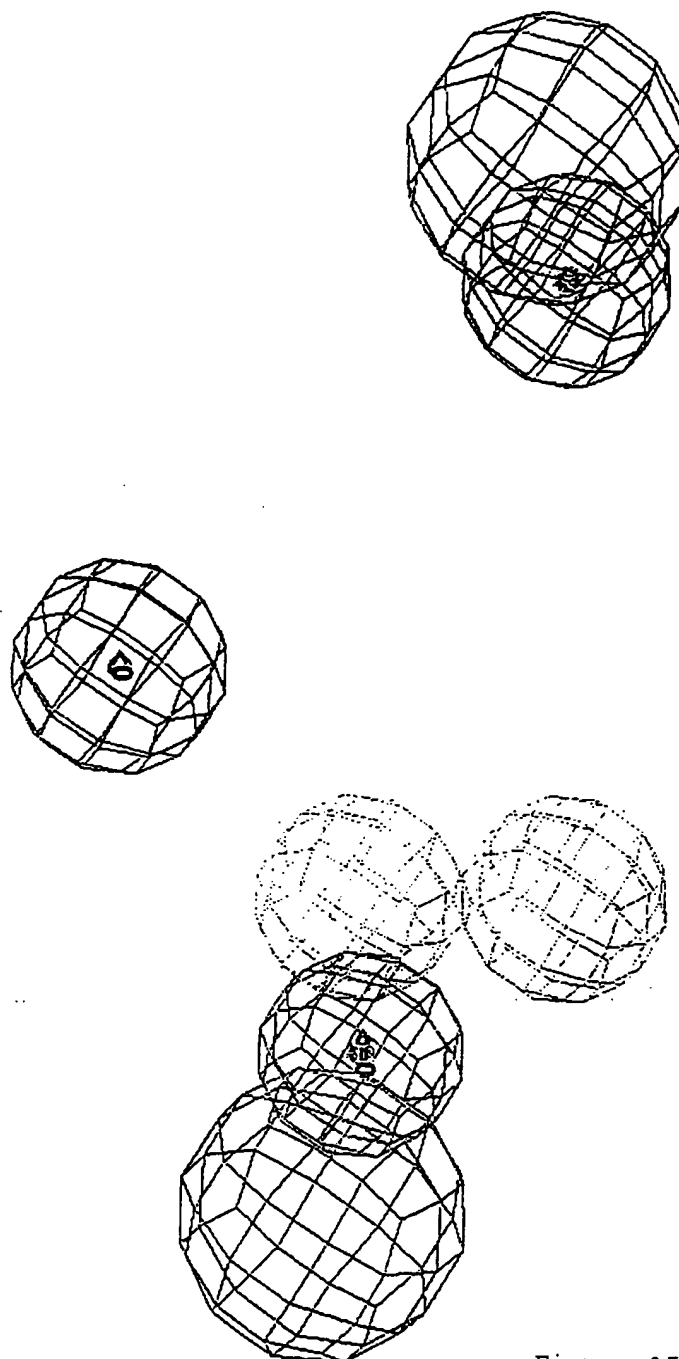
Figure 15

16/40
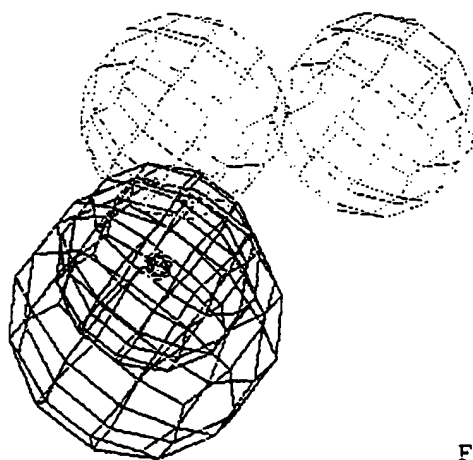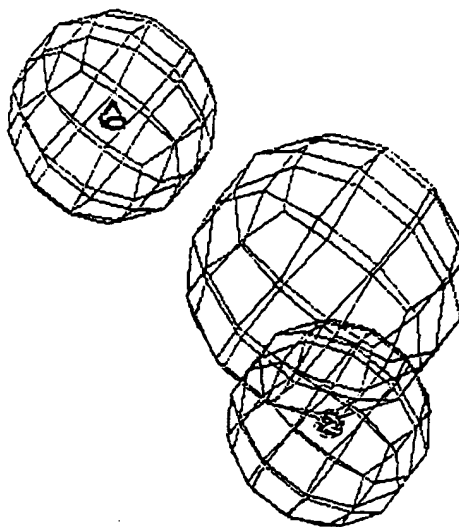
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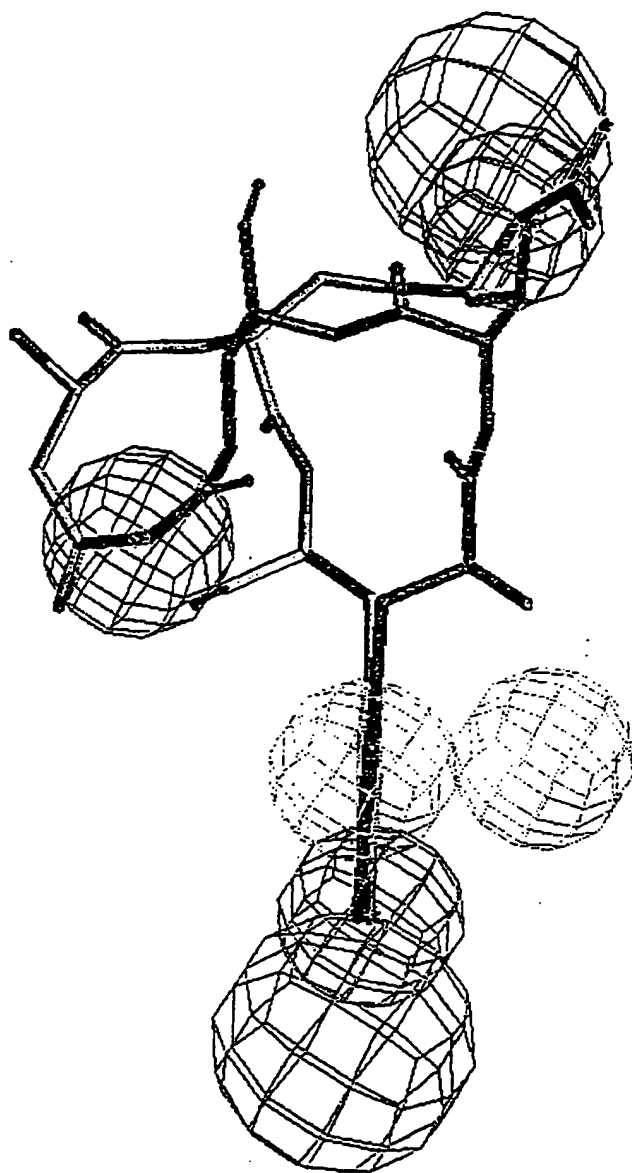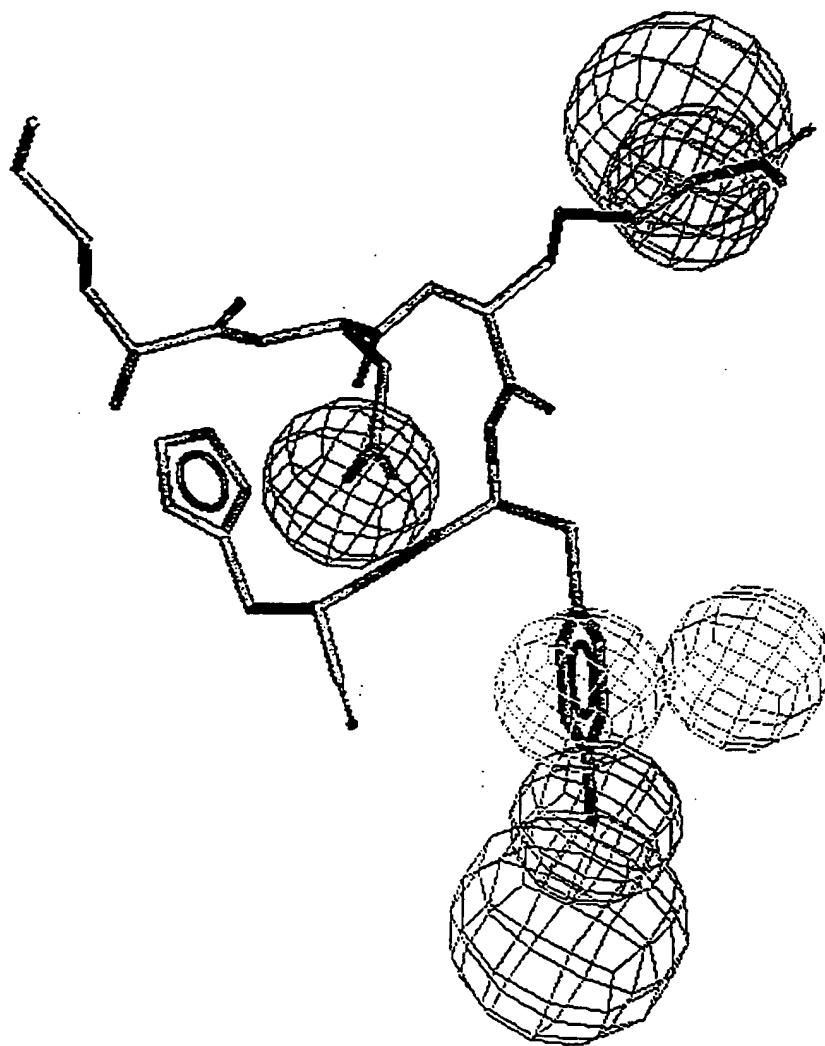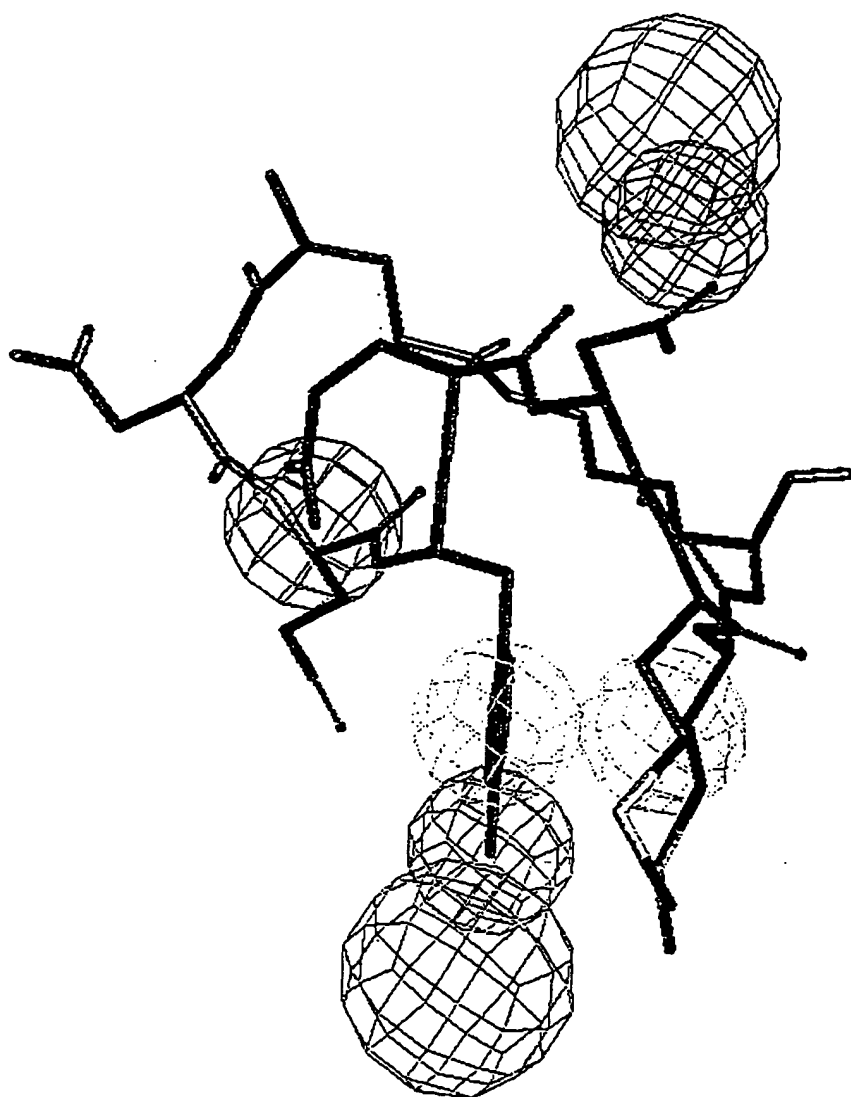
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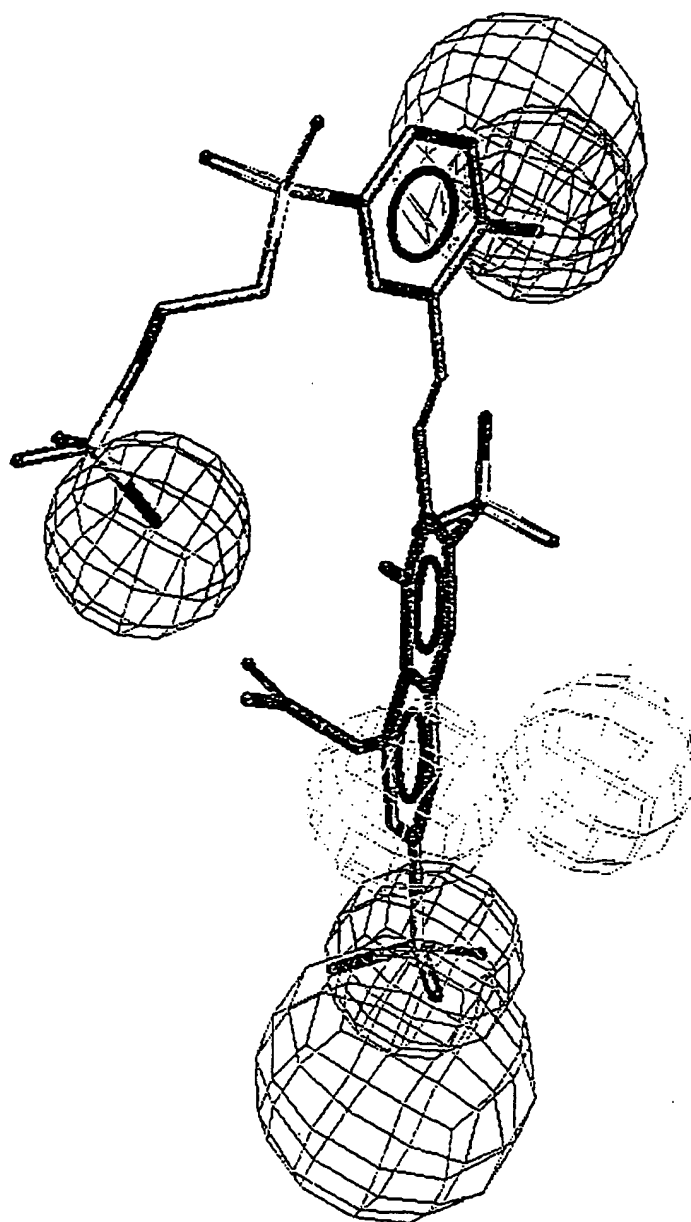
Figure 18

Figure 19
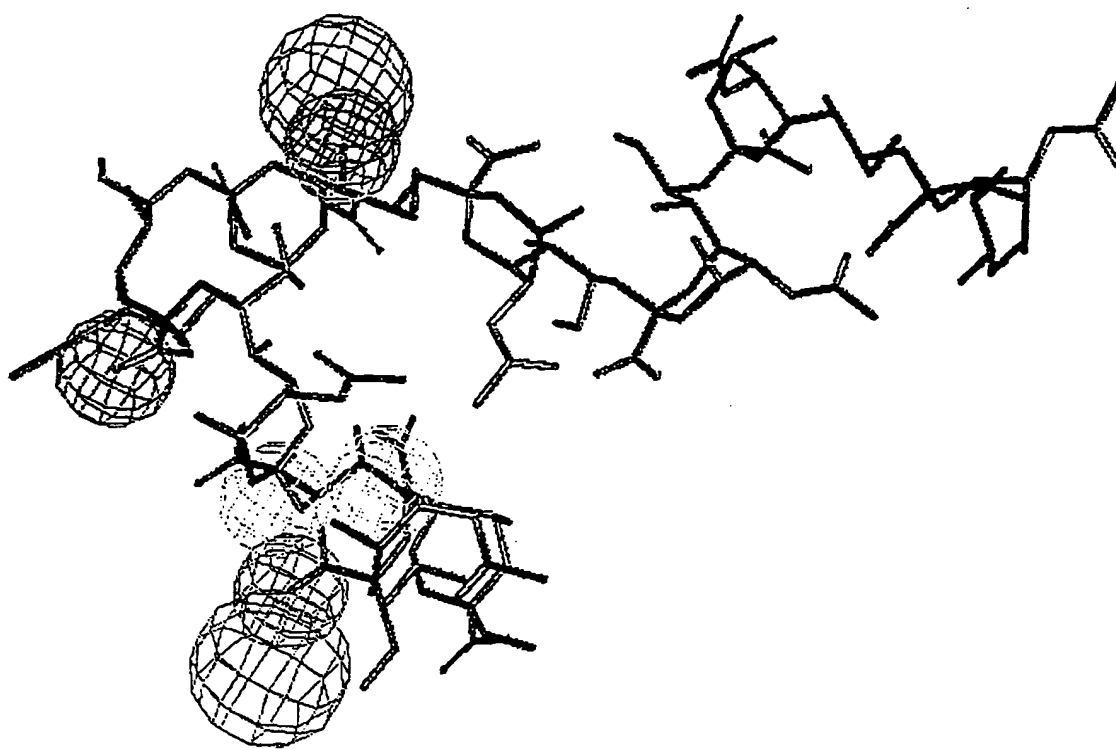
Figure 20

Figure 21
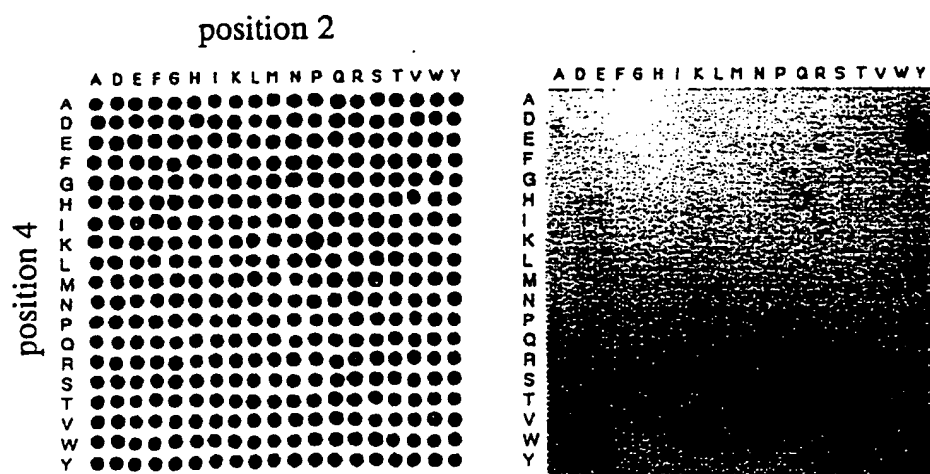
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library template = XOXOXXXX

Figure 22

Figure 23

Figure 24

8
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-0.859
-1.756

6
-0.100
1.844
-4.254

7
-0.753
0.333
-2.391

5
-0.394
-0.200
-2.048

4
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1.544
0.832

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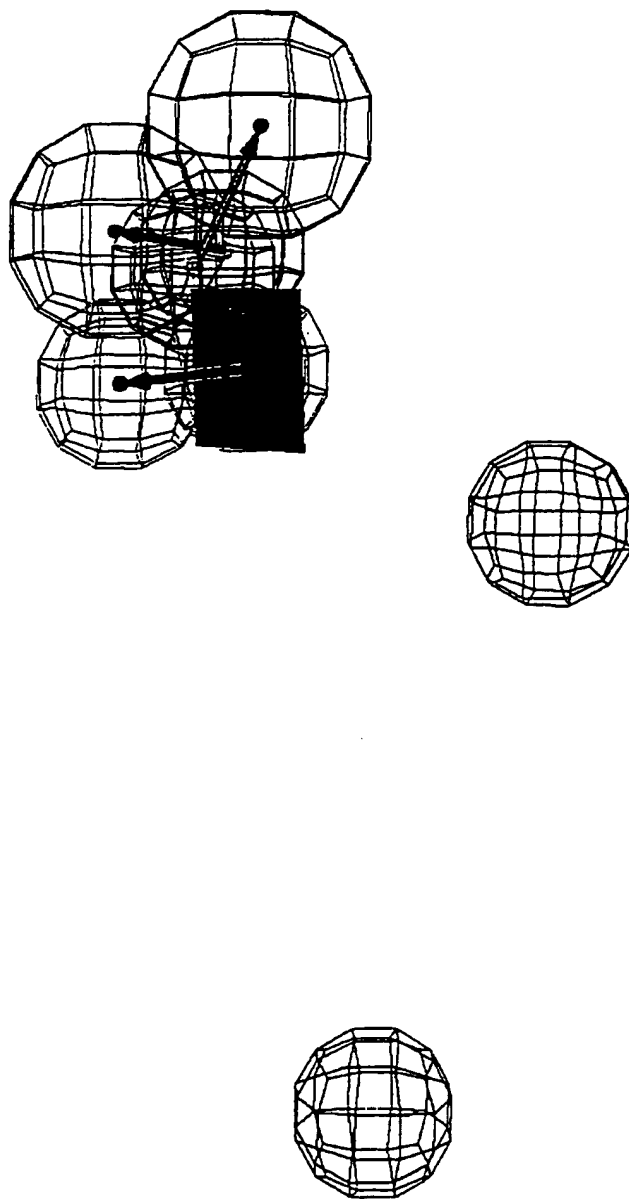
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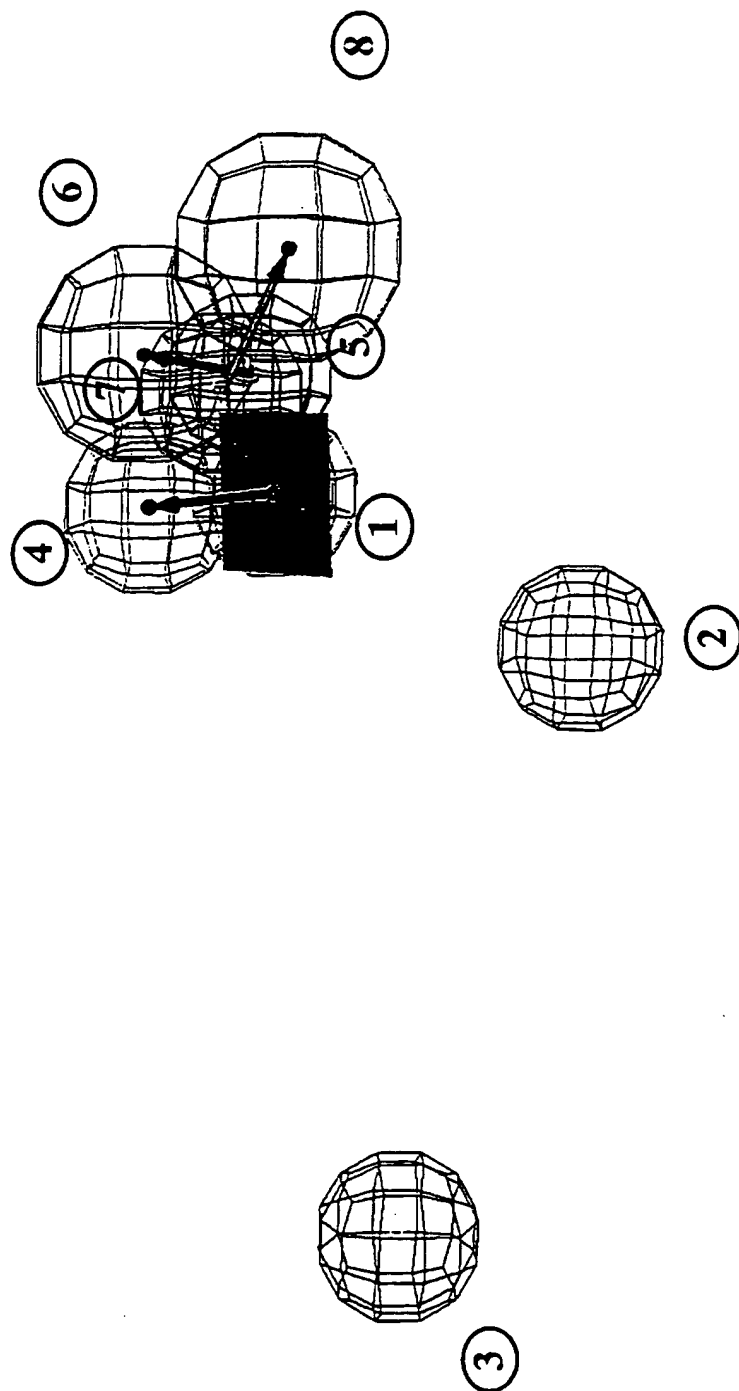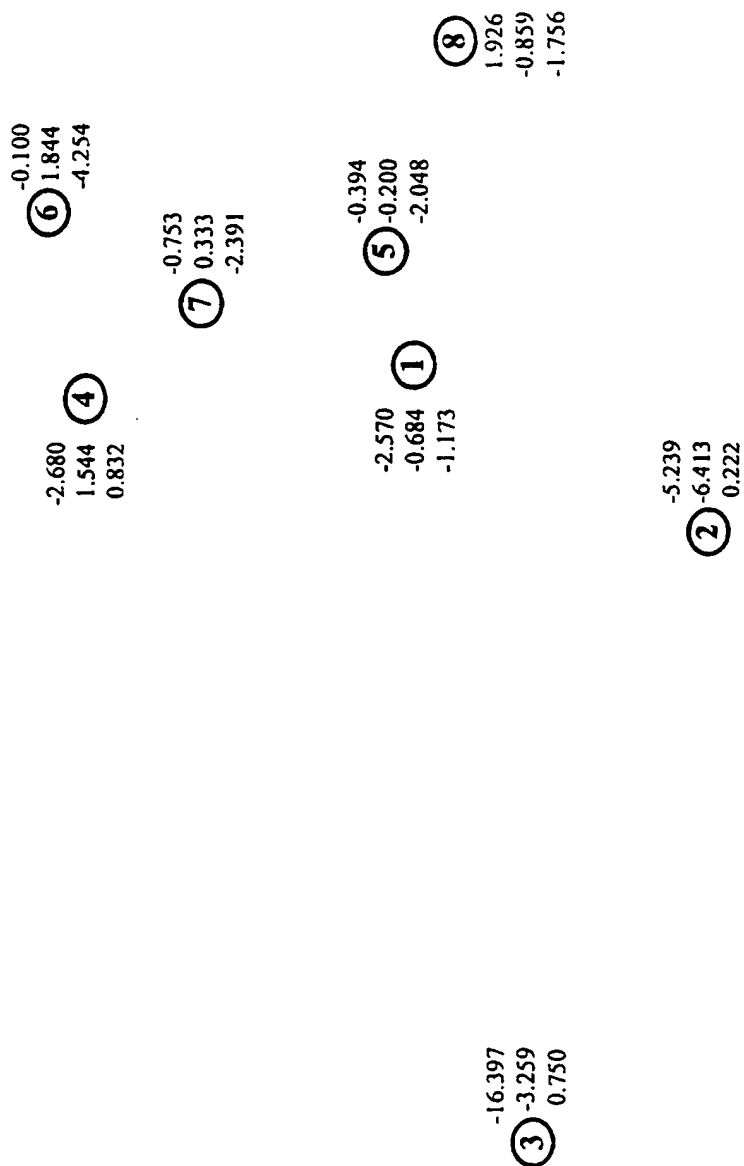
Absolute positions of each centroid in cartesian XYZ space

Figure 25
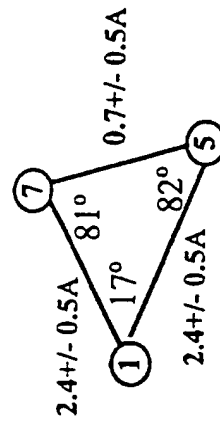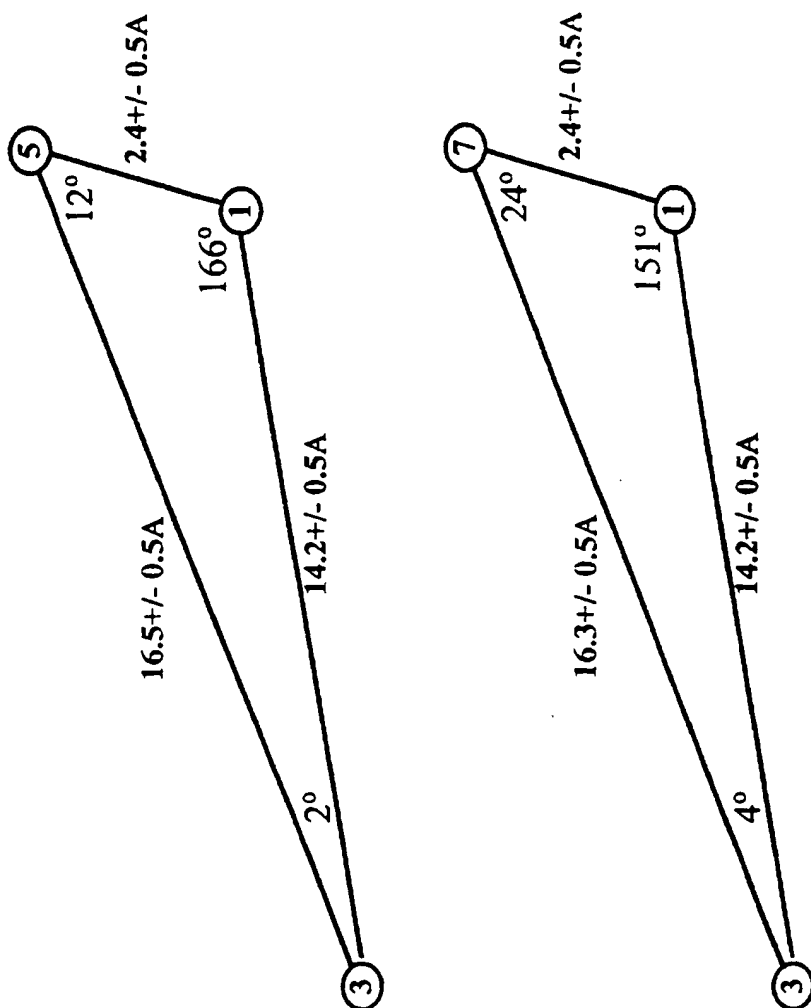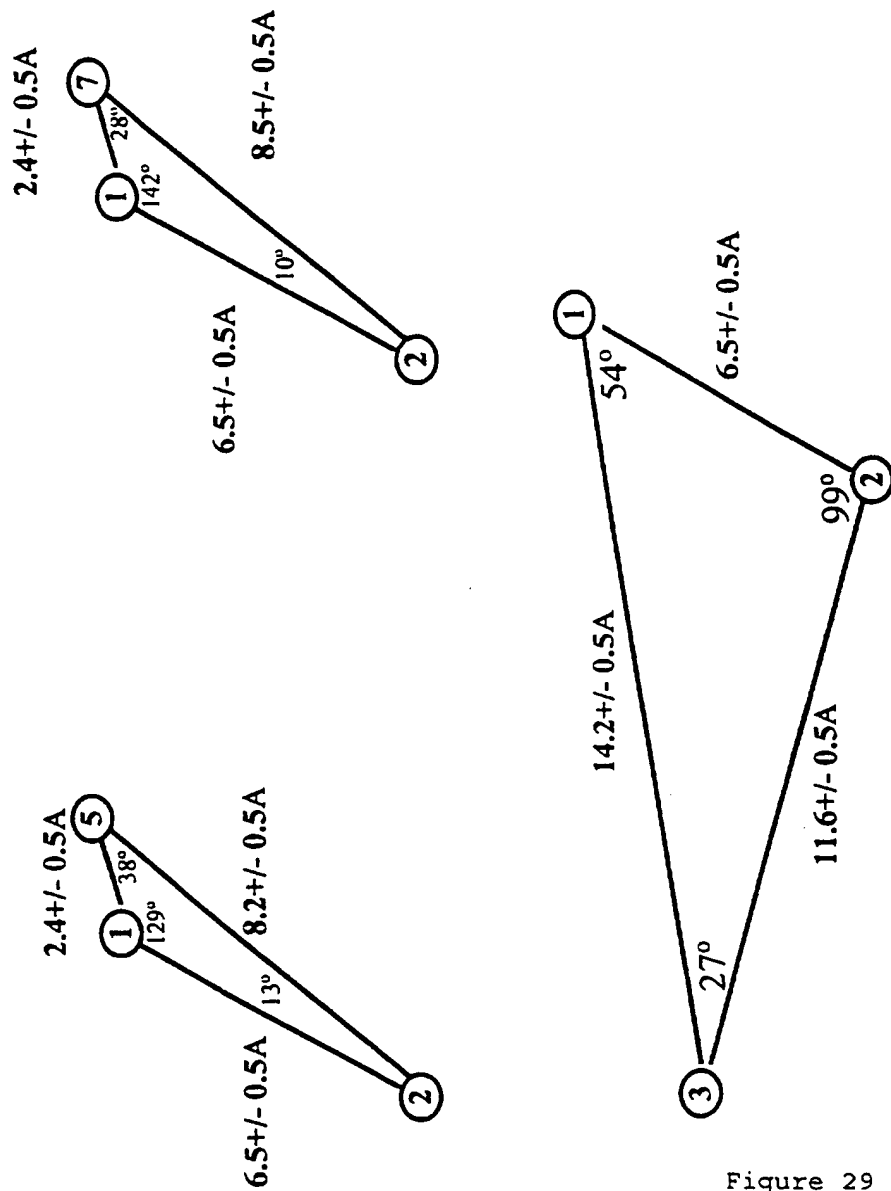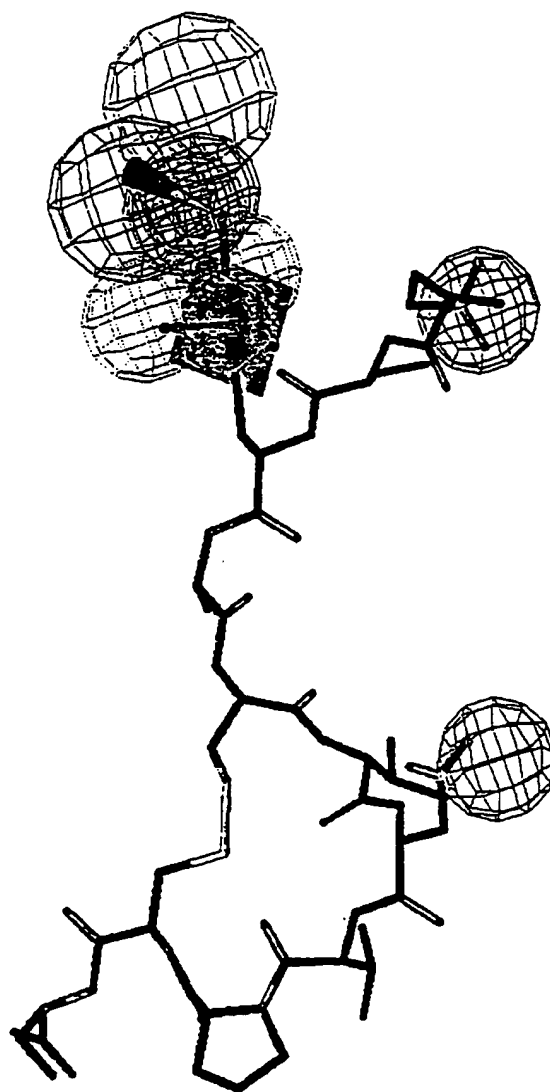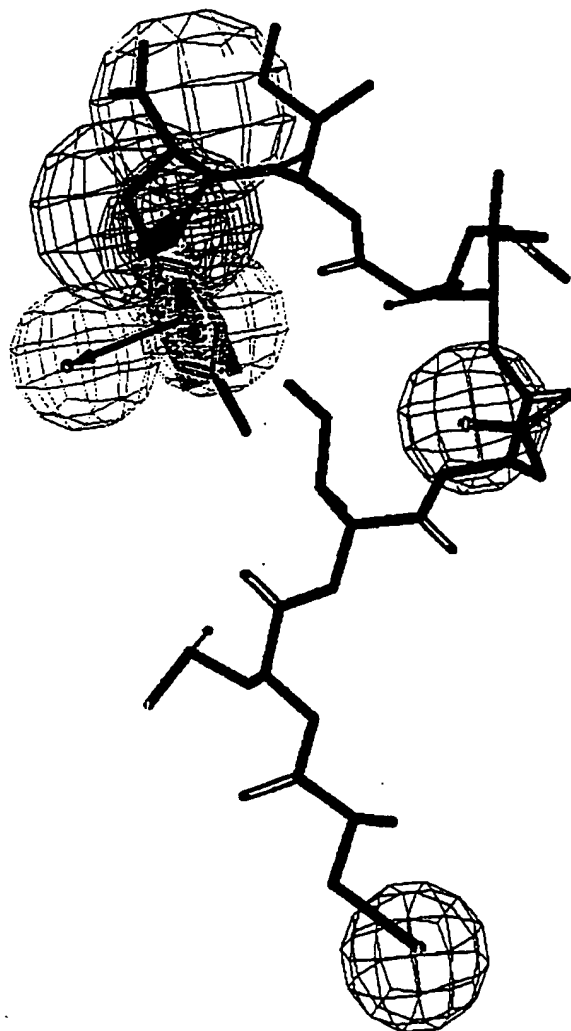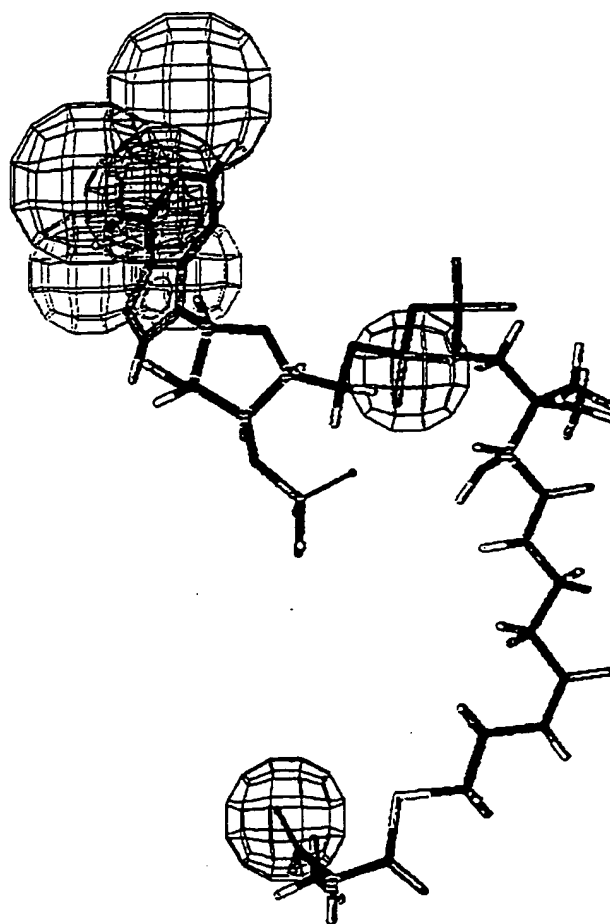
Figure 26

Figure 27
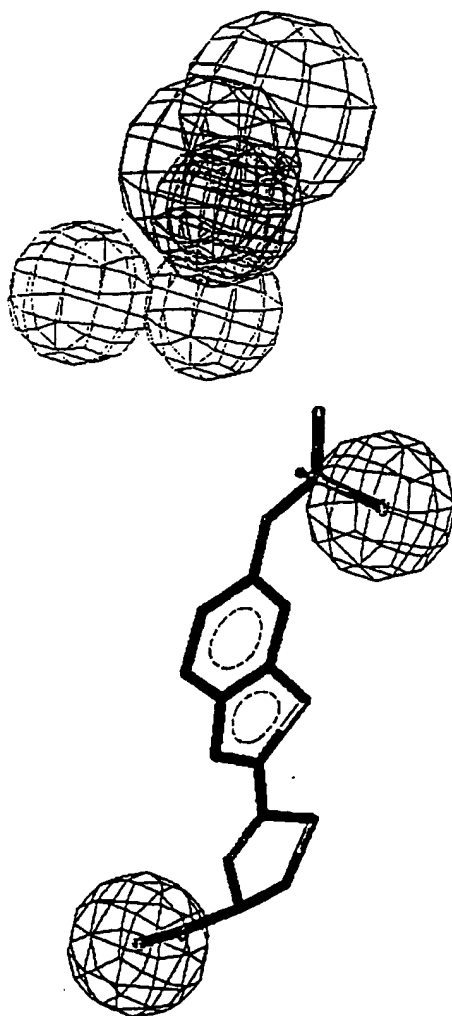
Figure 28
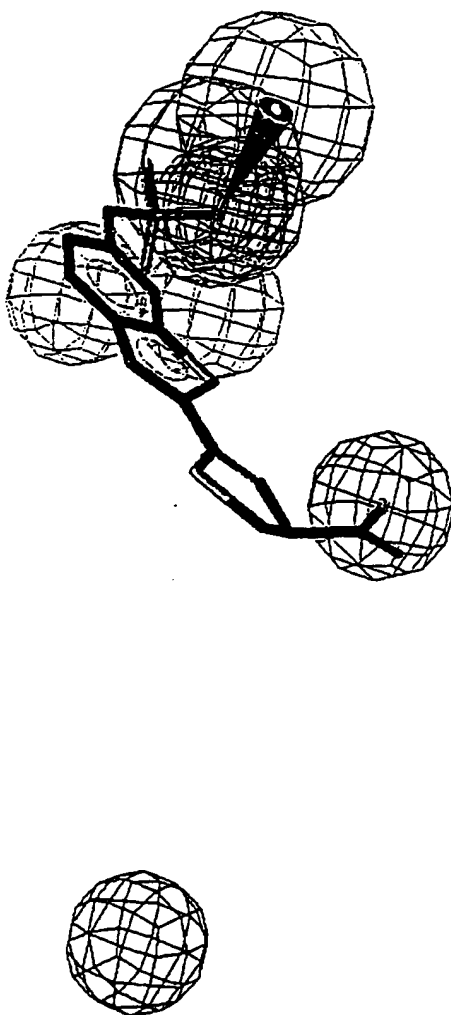
Figure 29

Figure 30
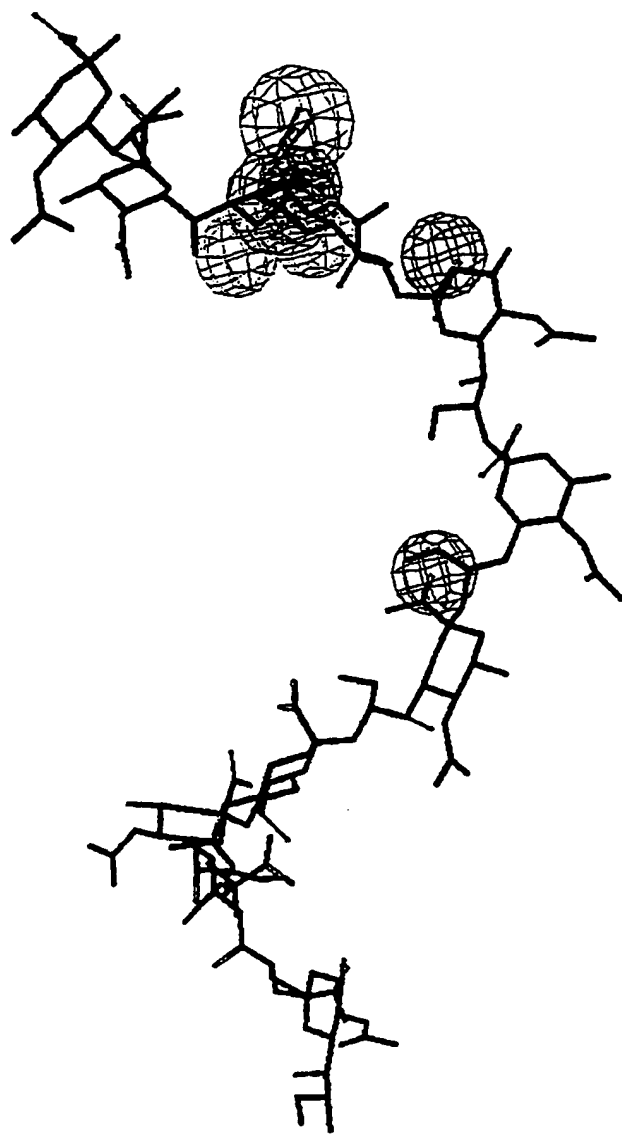
Figure 31

Figure 32

Figure 33

Figure 34

Figure 35

Substitution Net of Peptide GDN....

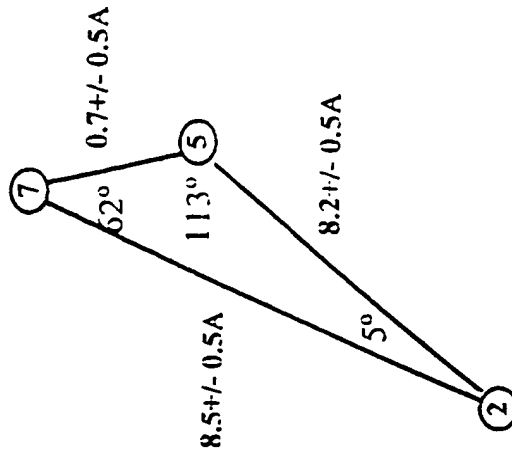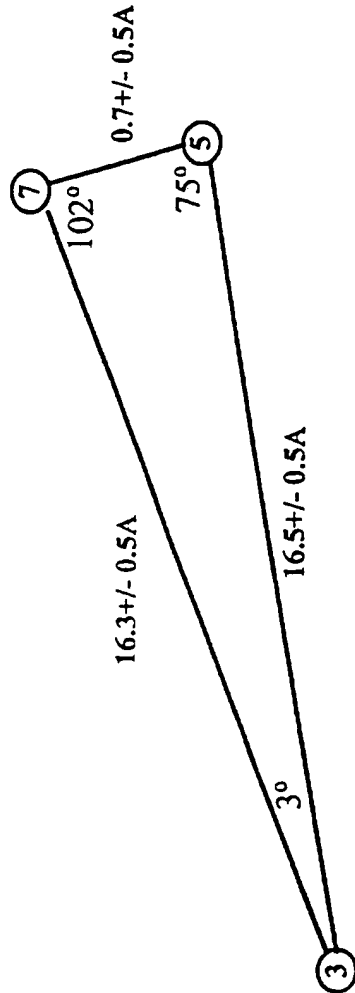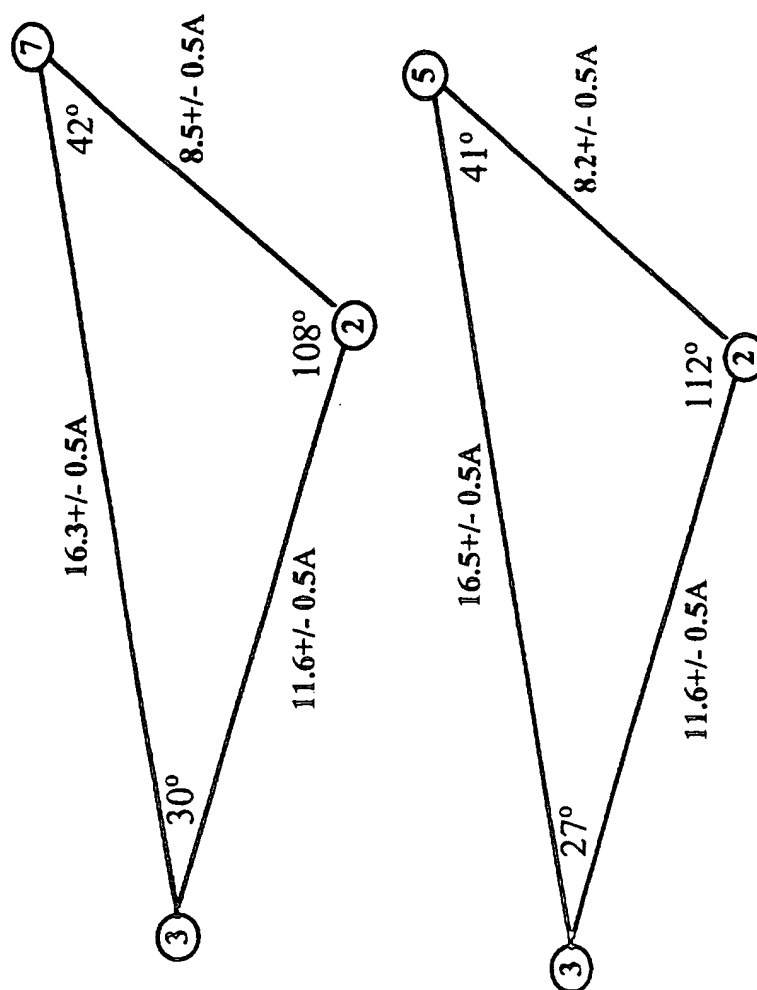|	A	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	comments
E	-2	$		-3	-1	-3	-3	-3	#	-3	-1	#	-2	#	#	-1	-3	-2	-2	D>E>T>others
S	-2	#	#				-1	-2	-2	-1		#	-2	-3	-2	-2	-2	#		
Y	2	#	#	#	-1	-1	#	-2	-2		#	#	#	-3	-2	-2	#	#	$	A>Y>G/H/M etc.
A																				
C	-2	#		0	0	0	0	0	-2	-3	-3	-2	#	#	#	#	$	#	#	V/E/F/G/H/L - (D,R,S,T,W,Y not tolerated)
V	0	#	#	#	#	#	#	#	#	#	#	#	#	.	#	#	#	#	#	D only, no substitutions tolerated
D	#	$		-2	#	#	-2	-3	#	#	#	$	#	#	0	-2	#	#		
T	-2	-2	-2	-2	#	#	-2	-3	-2	-2	-2	$	-2	#	0	-2	0	#	-1	P/S, all other substitutions suboptimal or not tolerated
P																				
C																				
S																				

Figure 36

List of Compounds Fitting the Combinational Pharmacophore

	CAS Number
Azosulfamide Disodium Salt	000133-60-8
Methylmalonyl Coenzyme A Tetralithium Salt Hexahydrate	104809-02-01
Beta-Nicotinamide Adenine Dinucleotide Phosphate Na-Salt	104809-28-1
Nicotinamide 1,N6-Ethenoadenine Dinucleotide Phosphate Sodium Salt	104809-28-1
Kayarus Supra Violet 5bl Conc	
Nicotinamide Hypoxanthine Dinucleotide Phosphate Disodium Salt	006739-64-6
P1,P5-Di(Adenosine-5') Pentaphosphate Sodium Salt	004097-04-5
Acid Violet 7	004321-69-1
P(1),P(5)-Di(Adenosine-5') Pentaphosphate Pentasodium Salt	4097-04-5
S-Methylmalonyl-Coenzym A Tetralithium Salt	104809-02-01
Acid Violet 5	010130-48-0

Training Set for the Combinational pharmacophore

| Tripeptide Sequence.	Relative affinity
TYR-THR-GLU	1
TYR-SER-GLU	3.3
ASP-ASN-TYR	100
TYR-THR-MET	200
TYR-THR-ASN	200
TYR-THR-GLN	200
TYR-GLY-GLY	1000
GLU-GLY-TYR	2000
ALA-GLY-TYR	3333
TYR-GLY-THR	10000
GLN-GLY-HIS	100000
PRO-GLY-HIS	200000
ASN-GLY-HIS	333333
SER-GLY-HIS	500000
HIS-GLY-PRO	1000000
HIS-GLY-ASN	1000000
HIS-GLY-SER	1000000
HIS-GLY-GLN	1000000
TYR-GLY-ALA	1000000

Figure 37

Replacement Net of Peptide TIP....

	A	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	comments
D	-1	$	1		0	#		#	#	#	#	#		-1	#		#	-2		D/E/I/H/W>A/S>Y
D	#	$	#		#	#	#	#	#	#	#	#		#	#		#	#		D only tolerated
E	-1	-1	$		#	#	-2		#	2	#		#	#		#	#		:	P>A/D/E>K, (H,N,S,V,W,Y - not tolerated)
I	#	#	#		#	#		#	#	#	#		#	#		#	#		I only tolerated
E	#	#	$		#	#	#	#	#	#		#	#		#	#		E only tolerated
V	#	#	#		#	#	#	#	#	#		$	#		V only tolerated
M	0	0	#		#	#	#	#	#	#		#	#		#	N/A
I	-1	0	-1		#	#	#	#	#	#		-2	-1	0		1	Y>A/I/D/W>V/E>S (H,K,N,P - not tolerated)
Y	-1	0	#		#	#	#	#	#	#		-2	0	0#	$	A/V/Y>S (D,E,H,K,N,P,W - not tolerated)

Figure 38
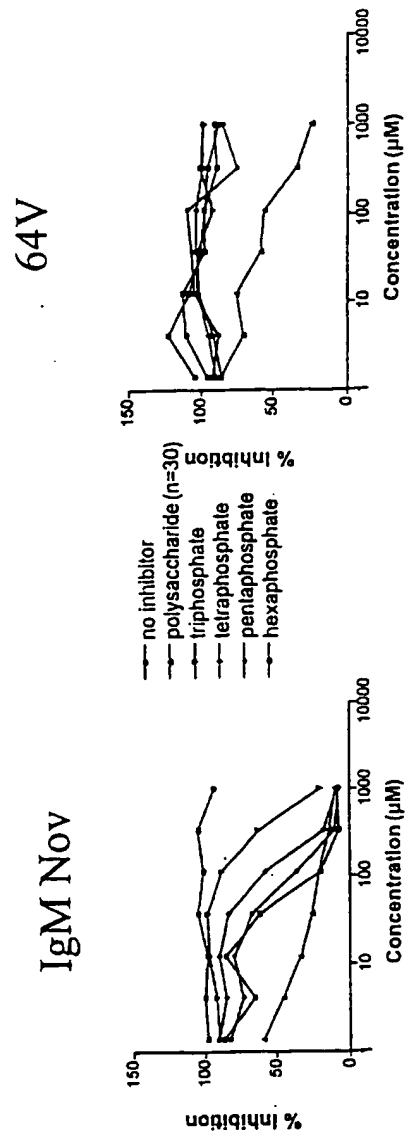
Figure 39

Binding of IgM Nov to
Ala-scanned mutants of GDN...

GDN FES YAC VDT PCS

Figure 40

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   C07K14/22   C07K7/08   C07K7/06   C07K16/12   A61K38/04
         A61K39/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   C07K   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLINICAL MICROBIOLOGY REVIEWS, vol. 7, no. 4, October 1994, pages 559-575, XP002039373 DIAZ ROMERO E.A.: "Current status of meningococcal group B vaccine candidates: capsular or non-capsular" cited in the application	1,53-55, 60-62, 65,67, 68,70, 71,73-75
A	see the whole document	2-52, 56-59, 63,64, 66,69,72

-/--

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 September 1997	1 9. 09. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 98, no. 9, 28 February 1983 Columbus, Ohio, US; abstract no. 65800, MEDVEDEV E.A.: "Effects of neuropeptides, modulators of memory, on background and induced electrocortical activity in rabbit cerebral hemispheres" XP002039375 see abstract & NEIROFIZIOLOGIYA, vol. 14, no. 6, 1982, pages 578-584, ---	1-8,11, 12,15, 18,19, 21,23, 26-31, 33,75
X	CHEMICAL ABSTRACTS, vol. 122, no. 13, 27 March 1995 Columbus, Ohio, US; abstract no. 161287, AGUILAR E.A.: "Molecular definition of the retention parameters of peptides separated by RP-HPLC" XP002039412 see abstract & PEPT.RES., vol. 7, no. 4, 1994, pages 207-217, ---	1-20, 23-27, 30,31, 33,75
X	CHEMICAL ABSTRACTS, vol. 119, no. 23, 6 December 1993 Columbus, Ohio, US; abstract no. 244157, CHO E.A.: "Substrate specificities of catalytic fragments of protein tyrosine phosphatases..." XP002039413 see abstract & PROT.SCI, vol. 2, no. 6, 1993, pages 977-984, ---	1-12, 14-21, 23-27, 30,31, 33,56,75
X	WO 90 06696 A (PRAXIS BIOLOG INC ;RIJKINSINSTITUUT VOOR VOLKSGEZ (NL)) 28 June 1990 The whole document; see esp. Table 8,seq 5 see claims 1-58 ---	1-7,39, 46-52, 56,59, 61-63, 65,66, 68,69, 71,72, 74,75

2

-/--

C.(Continuation) D CUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 26365 A (UNITED BIOMEDICAL INC ;WANG CHANG YI (US)) 5 October 1995 SEQ ID No. 26 ---	1-9,11, 13-16, 18-20, 23,24, 26,30, 33,40, 42, 46-48,75
X	WO 92 18150 A (ANERGEN INC) 29 October 1992 see page 21, line 36; claims 1-30 ---	1-8, 15-19, 21,40, 46-48, 56,75
X	WO 96 00238 A (PEPTIDE THERAPEUTICS LTD ;LEWIN IAN VICTOR (GB); BUNGY ALI GHOLAM) 4 January 1996 see the whole document ---	1-8, 15-19, 21,40, 46-48, 56,75
X	GB 2 282 380 A (MERCK & CO INC) 5 April 1995 See esp. SEQ ID NO. 10 ---	41, 46-51, 56,75
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 92, April 1995, WASHINGTON US, pages 4021-4025, XP002039374 WESTERINK E.A.: "Peptide mimicry of the meningococcal group C capsular polysaccharide" see the whole document -----	1-75

2

INTERNATIONAL SEARCH REPORT

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:
   Please see Further Information sheet enclosed.

2. [X] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out, specifically:
   Please see Further Information sheet enclosed (obscurities).

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all
   searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report
   covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   [ ] The additional search fees were accompanied by the applicar 's protest.

                                     [ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1992)

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/210

Remark : Although claims 63-68 are directed to a method of treatment of
(diagnostic method practised on) the human/animal body the search
has been carried out and based on the alleged effects of the
compound/composition.

Obscurities :

The scope of the claims 1-21,23-31,33-43,46-75 is unclear and speculative. The
claims 1-7 lack any clear indication concerning the structural features and size of the
compounds/peptides. The claims 8-21 and 23-31 and 33 define formulas that only
consist of variables or contain only one defined amino acid residue of the complete
sequence. Moreover expressions like "analogue and homologue derivatives thereof
by virtue of one or more amino acid addition, deletion, substitution" (claims 34-36)
cannot be considered to be clear and concise definitions of patentable subject-
matter (Art.6 PCT).
A complete search for peptides containing a trimeric motif as defined in the claims
39-41 would result in several thousands of documents and can therefore not be
considered to be meaningful.
Therefore a meaningful and economically feasible search could not encompass the
complete subject-matter of the claims. Consequently the search has only been
complete as far as related to sufficiently defined compounds and also to the actually
synthesised compounds and (closely) related analogs, that is the compounds
encompassed by the claims 22,32,44 and 45. (Art.17(2)(a)(ii) PCT).

Incomplete Search :
 Claims searched completely: 22, 32, 44, 45
 Claims searched incompletely: 1-21, 23-31, 33-43, 46-75

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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